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CROP LOAD CONTROL IN APPLE VIA SHADING: EXPLORING THE POTENTIAL OF CARBON UNBALANCES TO MANAGE FRUIT GROWTH AND TREE PERFORMANCE

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Esame finale anno 2010

A Federico

e a tutte le variabili

organiche e inorganiche

che da trent’anni modificano il mio stato di quiete.

-Zib-

Life can only be understood backwards

but it must be lived forward.

(Kierkegaard, S)

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Chapter I

CARBON BALANCE IN FRUIT TREES

INTRODUCTION

Carbon is the fourth most abundant element in the Universe by mass. It is present in all known lifeforms and in the human body is the second most abundant element. Carbon can be found in the atmosphere as carbon dioxide (CO_2) and in the Earth's crust too as the 15^{th} most abundant element (only 0.027) but it has a high impact on every biological process.

During the last decades, global warming concerns and related climate changes led to a renewed interest in CO_2 as the most important gas released from human activities.

Carbon dioxide such as other greenhouse gases (H_2O, CH_4 and N_2O) absorbs the energy at infrared wavelength causing an increased radiant heat reflection from earth surface.

In our planet there are 4 major districts where carbon accumulates or is released as a function of biological, geological and chemical processes: the hydrosphere, geosphere, atmosphere, biosphere (fig. 1). The hydrosphere (oceans and seas) and geosphere are the biggest CO_2 reservoirs even if only

a small part of this carbon is available and the migration process is very slow. The carbon dioxide concentration in the atmosphere is subject to annual variation due to the discontinuous photosynthetic flux by the plants. The biosphere is characterized by low quantities but extremely dynamic carbon compounds which have an important impact on all living things. By definition the carbon balance of an ecosystem at any point in time is the difference between its carbon gains and losses. The study of carbon balance in a district can make it possible to understand whether the reservoir is acting as a sink or a source of CO_2: plant uptake of CO_2 from the atmosphere is possible through the photosynthetic process; part of the fixed CO_2 can be released during the respiration process. Even dead tissues release CO_2 during decomposition . The carbon balance can be studied at the leaf or tree level, too (fig. 2). Modelling this balance allows us to better understand how the system works and for forecasting purposes.

By definition, a model is a simplified abstract view of a complex reality. A scientific model represents empirical objects, phenomena and physical processes in a logical way, using mathematical equations in order to understand the physiological process and in the end to be able to make forecasts about the results of the process. From input data, the system/model computes the value of the environmental variable(s) which describes the system state .

A well-known sentence by George P. Box (data) "*All models are wrong, but some are useful*", synthetically points out the good and the bad about modelization. Models are essentially abstraction, representation, reality simulation but they can greatly aid researchers in understanding the complex physiological interactions within the plant. First attempts at building simulation models were made in Agronomy, trying to identify and quantify ecosystem variables which have influence on annual plants . Others simulation models were built for springtime growth simulation, phenological phase or ripening date prediction, chill accumulation upon degree day calculations. Further studies led to incorporation into larger management models, adding different subunits in order to build more complex and detailed systems (eg.: net photosynthesis submodel, respiration submodel, resources partitioning, leaf area development, leaf abscission, environmental parameters, etc.). The existence of the model is strictly dependent on the presence of a database: data collection from field trials is a fundamental side of the work which generates the model itself and it should test the model too. Every model is characterized by a time-step, i.e. the time interval separating subsequent outputs of the simulation . The longer the time-step is, the less complex the simulation, but this will decrease the resolution.

Although in general one shouldn't expect differences between annual crop or perennial tree simulations (because the basic dry weight production and

partitioning can be considered very similar processes for both categories of plants), the lower structural and biological complexity of annual plants has helped a higher diffusion in annual crop simulation. Tree complexity depends on their perennial life cycle, their discontinuous and heavier canopy management (different training and pruning system), rootstock, cropping, absent or incomplete databases, among others.

The agricultural systems are basically photosynthetic systems and their productivity depends primarily from carbon assimilation and partitioning (De Wit, 1986). Modelling could prove particularly helpful in investigating tree productivity, source-sink balance, water relations, crop management effects, forecasting climatic change impact on crops, stress response evaluation, plant disease management, identification of environmental factors which cold reduce the growth of the tree, identification of carbon surplus/deficit and the impact on orchard productivity.

A challenging task for the modeller is to evaluate biomass production at tree or orchard level. In a fruit tree there is a wood perennial structure which is growing but also accumulates/exports carbohydrates and other reserves, with changing trends during the annual cycle. This makes it difficult to estimate the net biomass increase in fruit during the annual development and the harvest index (the fraction of dry weight allocated to fruit).

MODELS OVERVIEW

Apple Tree Simulator (R.C Seems et al., 1986)

This is one of the first models used to quantitatively describe whole tree physiological processes for apple tree growth, based on carbon assimilates production, allocation and utilization. The modelling work focused on three major aspects: tree-environment relations (which influence the end of dormancy and mediate growth rates); canopy structure and photosynthesis (a leaf spatial distribution model to simulate a wide range of canopy sizes and shapes); distribution and utilization of carbon assimilates by the tree organs for their growth and maintenance. For simplicity, tree water balance and mineral-nutrient status were not modeled, under the assumption of a non-limiting scenario for water and nutrients.

This tree model contained two subunits: the organ submodel focussed on tree respiration and growth by six main organ types (leaves, shoot, roots, fruits, stems and stored reserve carbohydrate); the physiology submodel which focuses on resource production, allocation and tree-environment interactions. Resource production is dependent on the photosynthetic process carried on by different types of leaves (spur/shoot), with a response that can simulate the low light condition of leaves located deeply inside the canopy. The simulation takes into account leaf age in the photosynthetic efficiency, increasing tissue respiration and decreasing the specific leaf weight as leaves naturally age.

If carbon assimilation is not enough, organs will relocate stored reserves; a borderline case is reached when portions of tree organs die in order to partition their reserves among other organs. Environmental input variables are average air/soil daily temperature, solar radiation and photoperiod.

Although the model program was coded in Fortran V, a Pascal version has been prepared. The command structure permitted the user to display and/or alter the 83 simulation variables at any time during the simulation process. The growing season can be modified by a set of 15 commands and the time needed to execute the one-year test program is about 30 minutes (on an early 1980's electronic processor).

Comparisons of actual and simulated biomass values were not always accurate, but the results from this complex simulator did show some correspondence to the real trees and as a rough approximation of tree growth this approach was very encouraging.

PEACH® (Grossman and DeJong, 1994)

The Peach® model simulates vegetative and reproductive growth of peach trees, based on the assumption that plants grow as collections of semi-autonomous, interacting and competing organs. The simulated carbon assimilation is dependent from solar radiation, minimum and maximum temperature, canopy light interception, leaf area index and photosynthetic rate. The partitioning module is based on sink strength, distance from source organs and available carbohydrates.

Sink strength for each organ type is a pre-determined, genetic plant feature: net sink strength is the maximum growth rate at which the organ can accumulate dry matter per unit of time and it is influenced by its proximity to the source. The potential net sink strength is the product of sink size (S_{SIZE}, g_{DW}) and potential sink activity ($S_{ACTIVITY}$, g_{DW} g_{DW}^{-1} day^{-1}):

$$S_{NET} = S_{SIZE} \times S_{ACTIVITY}$$

The potential sink activity is experimentally obtained, working on trees with different crop loads. The potential gross sink strength is the sum of S_{NET} and the respiratory losses of the growing organ:

$$S_{GROSS} = S_{NET} + R_g + R_m$$

where R_g is growth respiration (g_{DW} dd^{-1}) and R_m is maintenance respiration (g_{DW} dd^{-1}). The potential sink strength of an organ can be decreased by suboptimal environmental conditions, insufficient resource availability, or both. The conditional sink strength is the rate of deviation from the potential sink strength caused by environmental conditions such as temperature and water availability. The apparent sink strength is the rate of change from the conditional sink strength determined by resource availability.

PEACH® is a state-variable model, in which fruit, leaf, current-year stem, branch, trunk and root weight are the state variables, and minimum and maximum air and soil temperatures, degree-days and solar radiation are the

driving variables. The rate variables that characterize carbohydrate assimilation and utilization are derived from previous studies on photosynthesis, respiration and growth potential in peach trees. One major assumption made for this model is that trees are not under water or fertilizer limiting conditions.

The photosynthetic carbon assimilation submodel was modified from an annual crop growth model (SUCROS ’86 – Van Keulen et al., 1982). This system explicitly simulates total daily canopy photosynthesis over canopy depth and diurnal light conditions. The assimilation module of SUCROS was modified to account for the discontinuous canopy within a peach orchard using empirical data on the seasonal pattern of a daily light interception. The light saturated instantaneous photosynthetic rate is adjusted for the effect of air temperature, leaf age and leaf nitrogen concentration as a function of canopy depth. Leaf area index is calculated from simulated leaf weight.

Leaf maintenance respiration rates were estimated from previously determined leaf specific respiration rates by the mature tissue method (Amthor, 1989; Grossman and DeJong, 1994). Stem, branch and trunk maintenance respiration rates were estimated by the regression method. The specific respiration rate is the sum of maintenance respiration rate and growth respiration coefficient times the relative growth rate. The carbohydrate cost of daily growth is calculated as the sum of the

carbohydrate equivalent weight of the dry weight added by growth (sink strength) and the respiratory cost of that growth, this cost must be multiplied by the weight of dry matter added.

PEACH® simulates carbohydrate partitioning on a daily basis. Higher priority goes to maintenance respiration requirements, then the residual carbohydrates go to other organs with the priority: leaves, fruit, stems, branches, trunk and finally roots. The carbohydrate requirements for growth are satisfied based on the conditional net sink strengths of the growing organs and their proximity to a source. All carbohydrate partitioning is characterized in terms of dry weight gain, representing the weight of structural growth and carbohydrate storage reserves. Sink strength is calculated by grouping organs of the same type together, rather than making calculations for individual organs, although it is recognized that transport occurs to individual organs. Growth occurs at the potential rate if sufficient carbohydrate is available. If this is not possible, the fraction of potential growth that can be supported is calculated as the ratio of the carbohydrate available after maintenance respiration to the carbohydrate requirement for potential growth. Trunk growth is determined by calculating the ratio of the carbohydrate available after fruit, leaf, stem and branch growth to the carbohydrate cost of daily trunk growth potential. The daily carbon budget is balanced assigning remaining carbohydrate to root growth.

The seasonal pattern of simulated fruit growth on trees that were heavily thinned at bloom was similar to the seasonal pattern of fruit growth potential used to calibrate the model, except during the final week before harvest, when simulated individual fruit weights fell below the calibration and field values. Examination of the fraction of potential growth allowed by the model for heavily thinned trees indicated that carbon assimilation limited simulated growth during the final phase of fruit growth, resulting in an underestimate of final individual fruit weight (Fig. 3). Simulated leaf, stem and branch growth on defruited trees followed the calibration equations (Fig. 4) for growth potential. The simulated pattern of trunk growth was of lower magnitude than the calibration curve. The root weight accumulated by the model undoubtedly exceeds actual root growth because nutrient uptake, exudation, and fine root turnover are not modeled. However, the prediction that simulated root growth was reduced on trees with large numbers of fruits compared to defruited or heavily thinned trees is supported by numerous studies indicating that the presence of a fruit sink decreases annual root growth.

Simulation models are useful tools for integrating information about plant processes that are measured on time scales of seconds and minutes, such as photosynthesis and respiration rates, with data on processes that are measured over longer time intervals, such as reproductive and vegetative growth. This model accurately simulates the balance between carbohydrate

supply and aboveground carbohydrate demand and supports the hypothesis that plants function as collections of semi-autonomous, interacting organs that compete for resources based on their potential sink strengths.

L-PEACH (Allen M., Prusinkiewicz P. and DeJong T.M., 1994)

This model of carbon economy combines the carbon partitioning rules of PEACH with a more detailed model, in which the growth and function of each organ is modelled individually within an architecturally explicit model of canopy growth. L-System is an acronym for Lindenmayer-Systems, from the name of Aristid Lindenmayer (1925-1989), a biologist who first developed a variant of a formal grammar used to model the growth precesses in plants and other organisms.

The PEACH model almost entirely ignored the interaction between tree architecture and carbon allocation. In addition, each organ type was treated collectively as a single compartment, and thus all organs of the same type grew at the average rate for that organ. Because of those limitations, there was no potential to simulate differences in organ size or quality as a function of location in the canopy. It was also impossible to use this model structure to simulate the function of individual organs and capture the influence of their performance on patterns of carbon partitioning.

L-Peach overcame these limitations with a more detailed model of carbon economy which allows to take into account the structural and functional aspects of the modelled plant in an integrated fashion. The model is

formalized in terms of modules that represent plant organs. An organ may be represented as one or more elementary sources or sinks of carbohydrates. The whole plant is modelled as a branching network of these sources and sinks, connected by conductive elements.

The modules behaviour is controlled by a set of functions which can be graphically defined by the user. All the elements of the network may have a non-linear and time-dependent behaviour.

An analogy to an electric network is used to calculate the flow and partitioning of carbohydrates between the individual components. In this analogy, the amount of carbon corresponds to an electric charge, carbon concentration to electric potential, and carbon fluxes to actual flows. Daily photosynthesis of individual leaves is represented as an accumulation of charge. The plant model is interfaced with a model of light environment, which calculates the distribution of light in the canopy. The amount of available water is determined by user-defined functions that characterize water stress as a function of time. Available water influences the uptake of carbohydrates by various sink.

The amount of carbon accumulated set the organ growth and bud production rates of new metamers. If the carbon supply is insufficient, organs (leaves and branches) are shed by the tree. Thus, the development and growth of the branching plant structure (topology and structure) are closely coupled with the production and partitioning of carbohydrates. In

each simulation step, a mature leaf can both gain some carbon due to photosynthesis, and lose some due to respiration and export to other parts of the plant.

Function *f1* (fig.5) relates the rate of assimilation to the amount of carbohydrates (charge) already present in the leaf. A leaf cannot accumulate carbohydrates without limit and if there is no place for the charge to go, the accumulation in the leaf decreases or even stops.

Function *f2* (plot not shown) captures the relation between rate of assimilation and incoming light. It is an increasing function of light intensity, asymptotically reaching the maximum rate at high illuminations.

Given the charge accumulated in the leaf, its source strength (in electrical terms, its electromotive force) is determined by a third function, *f3*. This electromotive-like force is higher as the leaf accumulates carbohydrates (fig. 6).

The L-Peach model includes the following sink types: internodes (further decomposed into three distinct sinks related to elongation growth, girth growth and storage), young leaves, buds, fruits, and roots. The behaviour of stem elongation sinks will be described in more detail, to serve as an example of the general methods used in the model. The carbon flux that provides stem elongation is a product of three functions:

$$i = f_a(v) * f_b(q) * f_c(w)$$

function f_a states that the flow of assimilates into a sink depends on the voltage v at the point where the sink attaches to the tree. In biological terms, this can be thought of as the relationship between the concentration of sugar in the phloem where the sink is attached, and the rate at which that sugar can be unloaded into the sink. The elongation of stem is not an open-ended process, but will stop (for a given segment) when that segment reaches a mature length. Modelling stem elongation is thus handled by placing an upper limit on the total charge accumulated by a given segment, Function f_b defines an upper limit to the total charge accumulated by a given segment. According to this function, as a stem segment approaches its mature size it will take up less current, even if a high voltage is present at the point where that segment is attached. Function f_c describes the influence of water stress on the model. Its argument is an index of water stress, which ranges from one (the plant has all the water it can use) to zero (the plant has no water available at all). Given an input the model generates a dynamic visualization of the modeled tree and simultaneously quantifies and displays the output data selected by the user. These data may include global statistics, such as the overall amount of carbon assimilated and allocated to different organ types, as well as local data, characteristic of specific organs selected by the user. The user can thus evaluate, both qualitatively and quantitatively, how different parameters of the model influence the growth and carbon partitioning in the plant.

The model can be used to simulate the simultaneous interactions of multiple factors, including crop load, rate of fruit maturity, carbohydrate storage capacity, water stress, and how these factors can influence the growth and carbohydrate partitioning within a tree. L-Peach can be considered an L-system-based template for simulating complex interactions within trees, including growth, carbon partitioning among organs, and responses to environmental, management and genetic factors (fig. 7 – 8). A very innovative characteristic of the model is to consider both the structural and functional aspects of the modeled plant in an integrated fashion. The model is not yet calibrated to any specific tree, and many postulated mechanisms are hypothetical. Often there is not enough experimental data to provide a firm foundation for these mechanisms.

SIMPLIFIED DRY-MATTER PRODUCTION MODEL - Malusim (Lakso A.N. and Johnson R.S., 1990)

Sometimes research on modelling of dry matter partitioning and yield of apple trees leads to complex models, which risk remaining incomplete or inadequately described and tested. Too much complexity can and will turn a model into something usable only by the developer(s) (fig. 9). To circumvent this and other difficulties, this simplified dry matter production model has been developed with "Stella" dynamic simulation automatic programming language (ISEE Systems, Lebanon NH – USA) http://www.iseesystems.com). One of the key features of this model is that this

programming language can be used effectively by researchers who are not trained in computer programming. This feature greatly expands the potential use and testing of the model by a higher number of researchers. This kind of software reduces the time needed to modify any model component and also the simulation time. Another peculiar trait of Malusim is the 1-day basic time-step. Shorter time steps (i.e., hourly) can increase the resolution but reduce usefulness.

Daily Canopy Photosynthesis – the integral model chosen was that described by Charles-Edwards (1982) for daily gross photosynthesis rate per unit ground area allotted per tree (in g CO_2 m^{-2} day^{-1}).

$$P_{daily} = \alpha\ S\ h\ P_{max}\ [1- \exp(-kL)] / (\alpha\ k\ S + h\ P_{max})$$

where

α = leaf photochemical efficiency or quantum yield in $\mu g\ CO_2\ Joule^{-1}$ total radiation;

S = daily integral of total radiation on a horizontal surface in MJ m^{-2} day^{-1};

h = daylength in seconds;

P_{max} = rate of light saturated leaf photosynthesis in g m^{-2} s^{-1};

k = canopy light extinction coefficient;

L = leaf area index per total area allotted per tree.

The temperature effect on photosynthesis is included as a fractional reduction of P_{daily} estimated by a specific equation which reaches a maximum at about 28 °C and zero at 0 and 44 °C.

Leaf Area Development Submodel – Many studies on the rate of leaf area development on apple shoots have shown that there is a quite consistent relation with degree-day temperature accumulation (Johnson and Lakso, 1985 and unpublished data). The daily leaf area increment per shoot (in m^2) was defined as:

$$LA_{incr} = 0.00008\ DegDay_{4C}$$

using a 4 °C mean-base for the calculation of degree-days. Initial modelling was based on estimated times of shoot cessation derived from information on distribution of shoot lengths and leaf areas at the end of the season and the assumption that shoots all grew at the same rate, but for different durations. The "fraction of growing shoots" was estimated as a function of accumulated degree-days, and multiplied times the total number of shoots to give the number of growing shoots at any given time, which is then multiplied by the daily LA increment rate.

LAincr daily =

(LAincr/shoot) (#Growing Shoots) (Fraction of Growing Shoots)

Respiration Submodels

All respiration models are based on the exponential response of the respiration rate (R) to temperature expressed by:

$$R = a\ e^{kT}$$

where a = R at T = 0 °C; k = temperature coefficient of R (the slope of lnR versus T); and T = temperature in degrees C. Individuals tissue submodels

for leaves, fruits, perennial structures were developed based on different *a* and *k* coefficients from the literature. Respiration rates for leaves and wood are based on surface areas, while fruit respiration is based on fruit fresh weight.

Required Inputs

Physiological processes that can reasonably be predicted (photosynthesis, respiration and leaf development rates) are driven by environmental inputs like max and min temperatures, total radiation and daylength. Other processes such as budbreak, termination of shoot growth, fruit set and fruit development are much less consistent. Thus, empirical inputs of shoot numbers/tree, fraction of shoots that are actively growing, fruit numbers, fruit weights and maximum P are required. These inputs may become submodels as our understanding of the regulation of such processes increases. At this time, though, it is more accurate to measure them than to model them.

The simplified daily integral approach to dry matter production has given very reasonable simulations of leaf area development, total dry matter produced and respiratory losses. Such simulation helped us to understand the variation in response to chemical thinners, that seems to be linked to particularly good or poor simulated carbohydrate supply and demand trends.

Whenever great simplification are made in a model of a complex system, many limitations occur. Unfortunately many processes are unmodeled because too little is known about some process.

The model is designed to help integrate many parameters realistically to show patterns of tree behaviour that may be helpful for our understanding (Lakso et al. 2006).

FIGURES

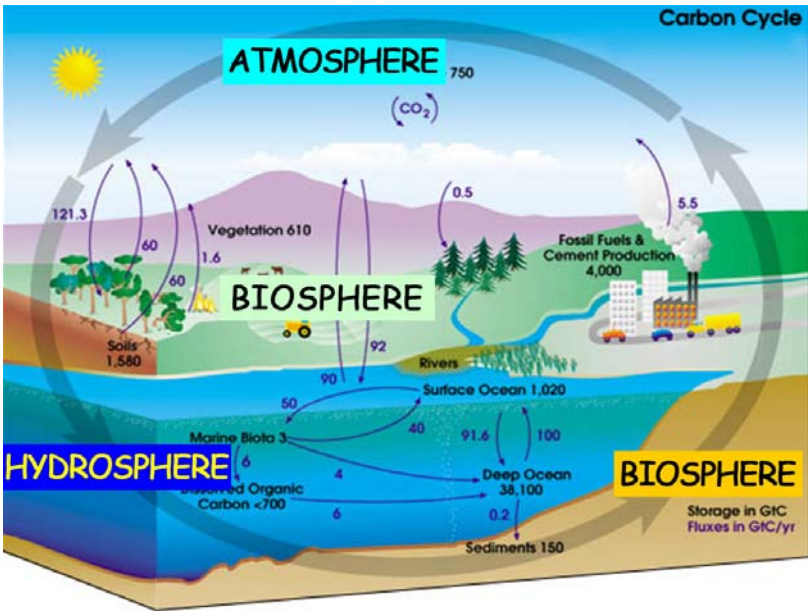

Fig. 1. The four major district where carbon accumulates.

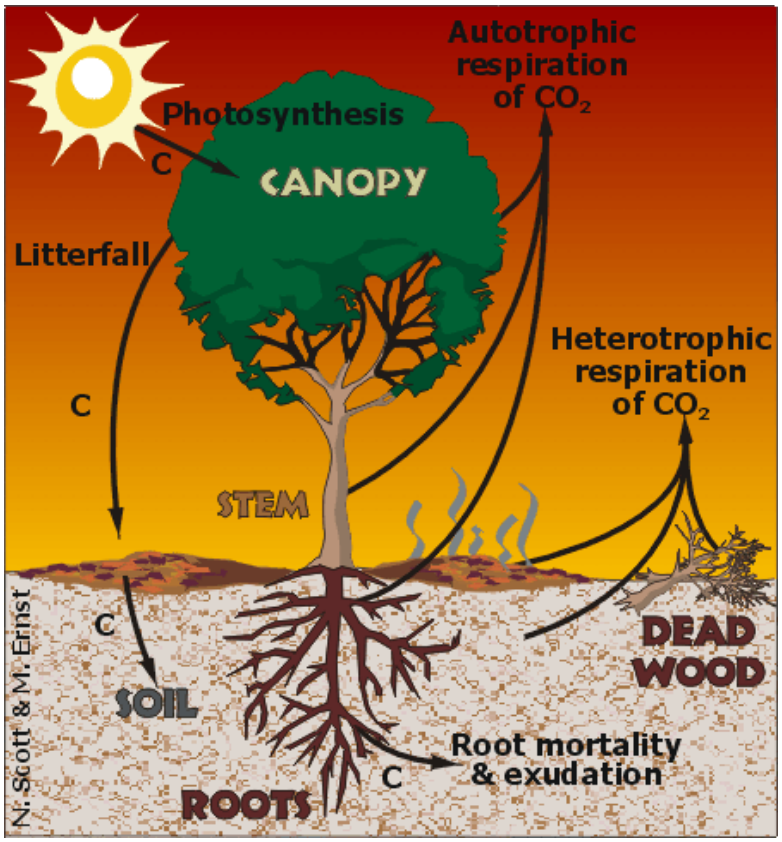

Fig. 2. Components of the carbon balance in a tree and its environment.

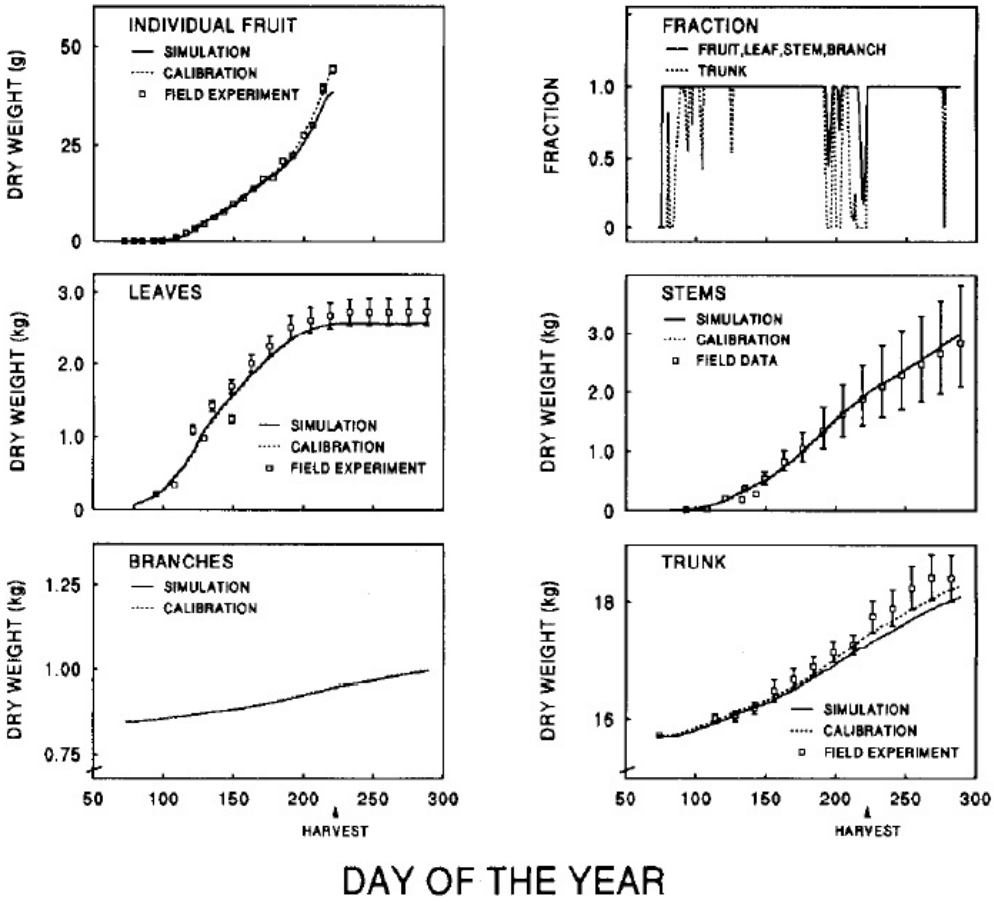

Fig. 3. Simulated and experimental seasonal patterns of organ dry weight per tree under calibration conditions.

Fruit

$$FWT = \exp(-7.39 + 0.032DD - (3.81 \times 10^{-5})DD^2 + (1.65 \times 10^{-8})DD^3 - (DD>700)(1.44 \times 10^{-8})(DD\text{-}700)^3 - (DD>1400)(2.73 \times 10^{-9})(DD\text{-}1400)^3)$$

Leaf

$$LWT = \exp(8.84 + 0.0091DD - (7.94 \times 10^{-6})DD^2 + (2.49 \times 10^{-9})DD^3 - (DD>1000)(2.38 \times 10^{-9})(DD\text{-}1000)^3 - (DD>2000)(2.87 \times 10^{-11})(DD\text{-}2000)^3)$$

Stem

$$SWT = \exp(1.61 + 0.011DD - (8.56 \times 10^{-6})DD^2 + (2.55 \times 10^{-9})DD^3 - (DD>1000)(2.32 \times 10^{-9})(DD\text{-}1000)^3 - (DD>2000)(1.21 \times 10^{-10})(DD\text{-}2000)^3)$$

Branch

$$BWT = \exp(844 + (5.50 \times 10^{-5})DD)$$

Trunk

$$TWT = \exp(15721 + (5.04515 \times 10^{-5})DD)$$

Fig. 4. Calibration equation for different organs. DD represent degree-days after bloom.

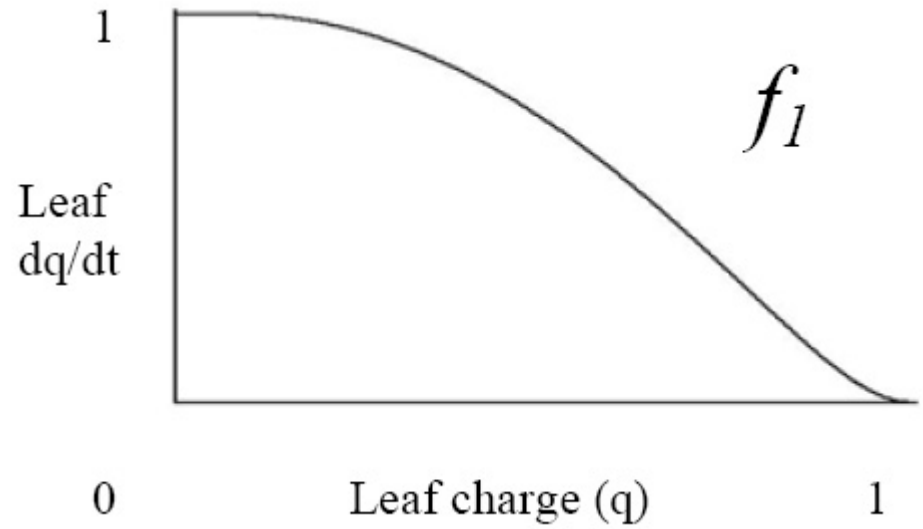

Fig. 5. Function f_1 relates the rate of assimilation to the amount of carbohydrates (charge) already present in the leaf.

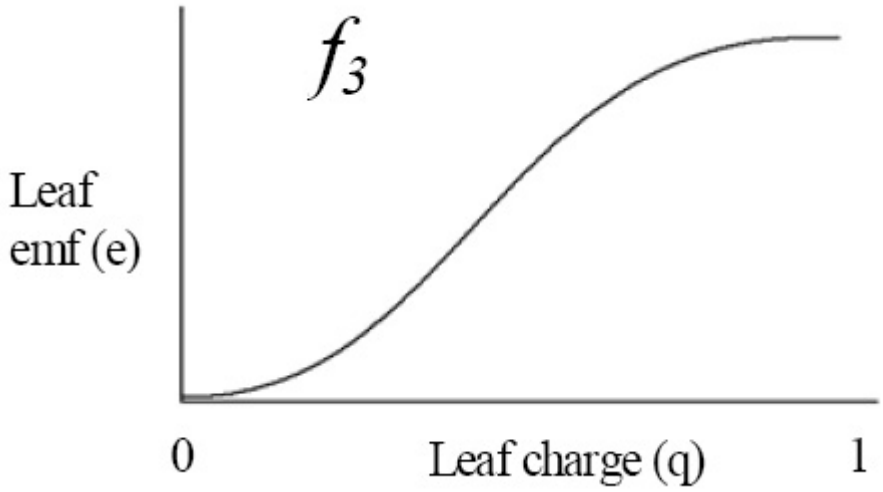

Fig. 6. Function f_3 relates the electromotive force of the leaf to the leaf charge in the leaf.

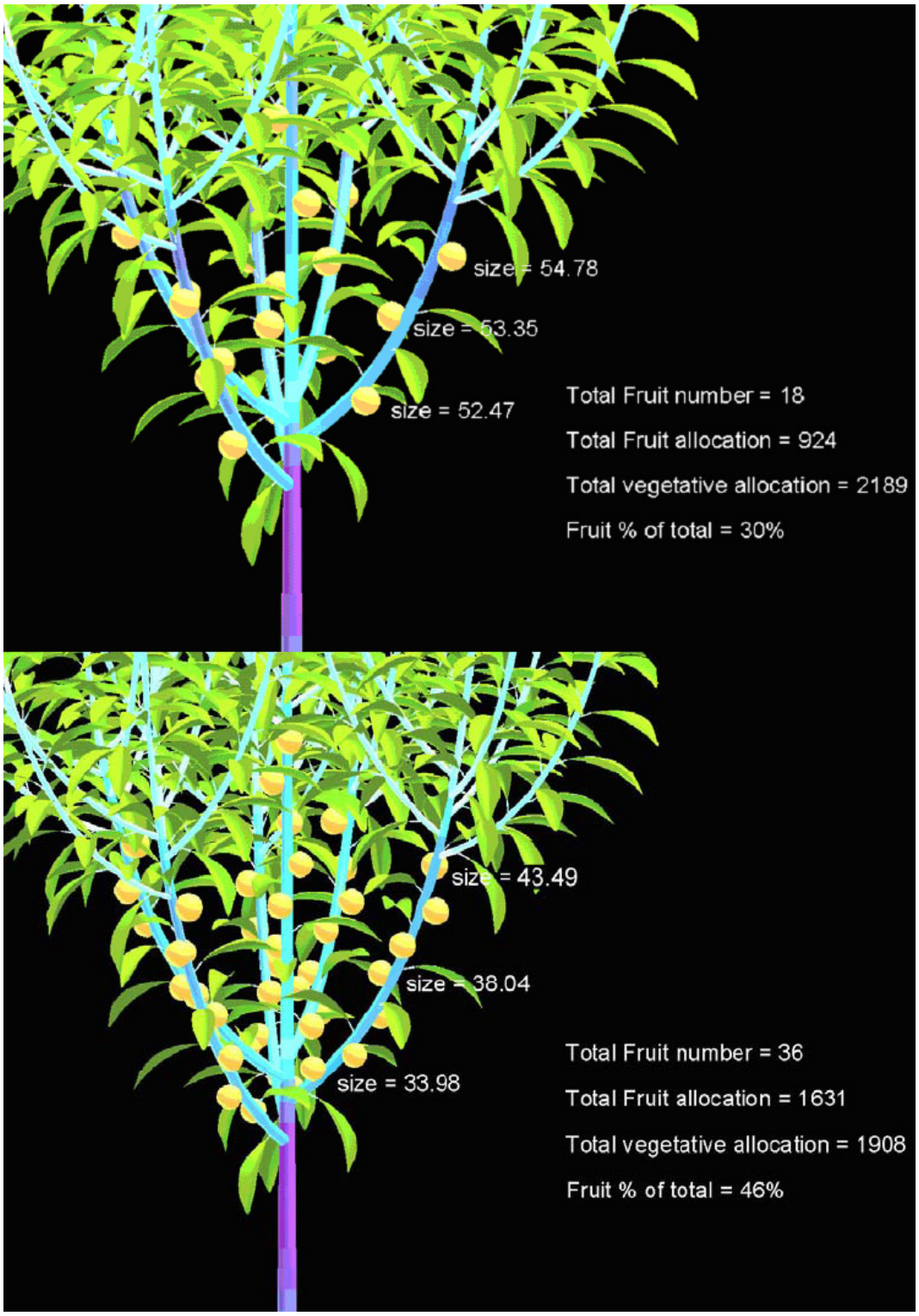

Fig. 7. Screenshot of the simulation program. The upper panel is the result of a simulation with heavy crop load and the lower is a simulation with half as many fruit.

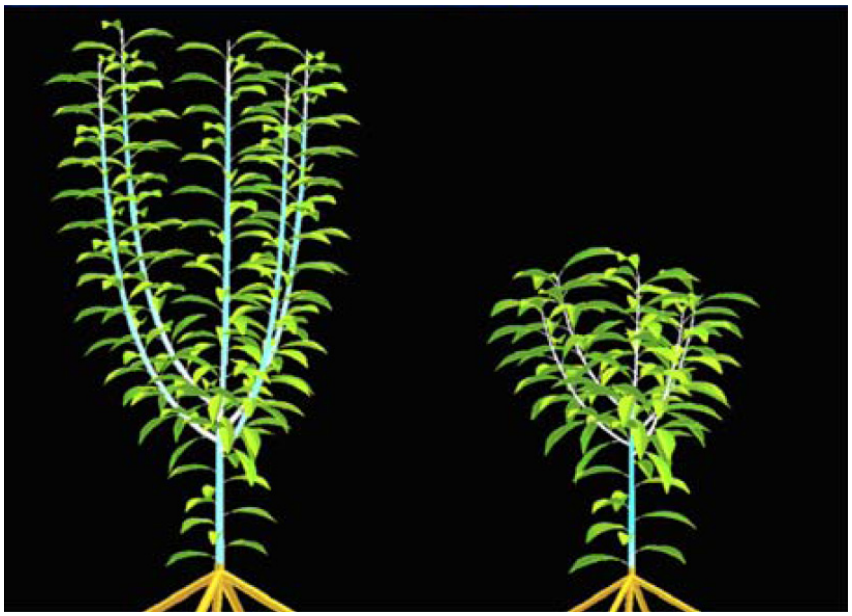

Fig. 8. This figure demonstrate the potential of the model to simulate the effects of irrigation frequency or mild water stress on tree growth. The tree on the left was simulated under conditions of full irrigation whereas the tree on the right experienced mild water stress during growth.

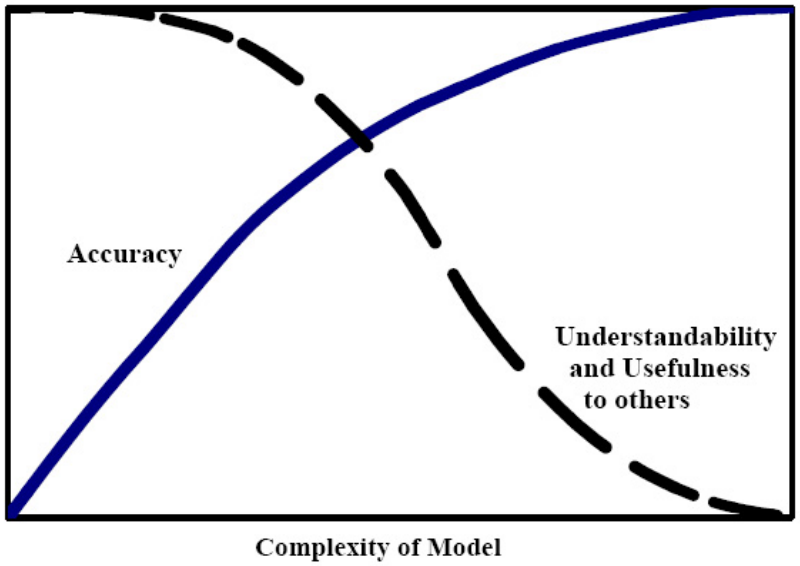

Fig. 9. General diagram indicating that as models increase in complexity from the most simple (e.g. single driving factor) the accuracy of predictions may increase, but the understandability of all interactions and regulation of the model decreases. Finding an appropriate balance is a challenge with all models, but especially with crop models in natural environments.

Chapter II

THINNING APPLES VIA SHADING: AN APPRAISAL UNDER FIELD CONDITION

INTRODUCTION

To satisfy market demand, and to attain profitable fruit production, apple growers must produce fruit of maximum quality, while retaining the highest possible yields. The conflicting nature of these two goals requires accurate management of tree crop load. Excessively low crop loads will lead to reduced productivity, despite a larger fruit size, with too many fruit, the yield per tree and per hectare will be increased, but fruit size will be reduced. In addition, excessive crop loads can result in alternate bearing in many apple cultivars (Jimenez and Diaz, 2004). Successful fruit production is achieved with high fruit numbers per tree at fruit set, followed by thinning to reduce that number to the optimum level determined for each tree and cultivar. Thinning is therefore one of the most important orchard management techniques used to improve crop yield and quality in apple (Link, 2000; Byers, 2003).

Thinning can be achieved by hand-removal of fruit, which is expensive, or *via* the application of phytochemicals which cause fruit drop. Several compounds have been tested over the years, and some have found wide commercial application in apple orchards. Apple responds best to chemical thinning although, almost invariably, it requiring hand-thinning adjustment to optimise fruit loads. Despite its wide use, chemical thinning of apples has retained a degree of uncertainty in the results achieved and, despite efforts to standardise application conditions, many experiments have shown substantial components of unaccounted-for variation (Jones *et al.*, 2000). Many factors contribute to variable outcomes of thinning, as the thinning response depends on complex interactions between temperature, shade, drying rate, and the physiological condition of the tree (Byers *et al.*, 1990; Dennis, 2000).

Schneider (1975) and Byers *et al*. (1985; 1990; 1991) reported several experiments in which fruit drop was induced by shading apple trees for a few days, at a specific period post-bloom (approx. 30 d after full-bloom; DAFB), and with a large reduction (≥ 70%) in the availability of light. Schneider attributed this effect to increased competition for photosynthetic resources between growing shoot tips and fruitlets, with the reproductive organs at a disadvantage at this early stage of development. Byers *et al*. (1985) provided evidence to support this hypothesis, replicating the results

of shade cloth by the application of Terbacil (DuPont Agricultural Products), a photosynthesis-inhibiting herbicide.

Corelli Grappadelli *et al*. (1994) showed that shading of apple branches caused a reduction in the amount of carbon partitioned to young fruitlets, as more carbon was drawn to the growing shoot tips during the first 5 weeks after full bloom (WAFB) in heavily (90%) shaded apple branches. The fruit drop caused by shading could be diminished and reversed by injecting sorbitol solutions into the tree trunk (Corelli Grappadelli *et al*., 1990). Further work has confirmed that, during the period from budbreak to approx. 30 DAFB, there is a net loss of carbon reserves, corresponding to the time when trees are most susceptible to artificial or environmental fruit loss by shading (Byers *et al*., 1991; 2003). Data are lacking, however, on the size of the decrease in photosynthesis that accompanies fruit abscission.

The fate of fruitlets seems to depend on their rate of growth. Zucconi (1981) showed that populations of growing fruitlets exhibited a bi-modal distribution, with slower-growing fruit representing the majority of those destined to abscise. Similarly, growth during the early stages can set the potential for larger fruit size at harvest. Lakso *et al*. (1989) reported a larger size at harvest in fruit on thinned branches, even though these fruit showed only a briefly higher relative growth rate (RGR) at 5 WAFB. Observing the growth of fruit over short intervals is difficult because the changes may be too small to detect using simple callipers, therefore little is known about the

effects of shading on fruit growth dynamics in the days preceding abscission.

To be able to transfer this approach to commercial practice, a method is needed to determine when to stop the shading treatment, in order to achieve optimum thinning, but not over-thinning. Musacchi and Corelli Grappadelli (1994) suggested an approach based on daily recordings of the pattern of fruit abscission. They removed the shade cloth immediately after detecting a sharp increase in the daily rate of fruit drop. This approach, however, has led to over-thinning in alpine areas of apple growing in Europe (e.g., South Tyrol and Switzerland; Kelderer *et al*., personal communication; Widmer *et al*., 2007), thus showing the need for a less empirical approach.

This paper reports on a study on the effects of shading on whole tree photosynthesis, daily and seasonal fruit growth, and on fruit retention in apple (*Malus* x *domestica* Borkh.) cultivar 'Imperial Gala' in the Po Valley of Italy.

MATERIALS AND METHODS

Plant material

The trial was carried out in an experimental orchard at the University of Bologna Experimental Farm, located in the south-eastern Po Valley (44º30'N; 10º36'E; 27 m a.s.l.) in 2007, on 12-years-old trees of 'Imperial Gala' apple on M.9 rootstock. Trees were trained as free spindle and

planted at a density of 2,381 trees ha^{-1} in a North-South orientation in a clay-loam soil. The orchard was managed following standard fertilisation, irrigation, and pest-management practices.

Experimental

Twenty-eight trees were selected for uniformity of blossom and divided in two groups of 14 replications per treatment, using single trees as replicates. The two treatments were thinned using chemicals (control) or artificially shaded. A neutral polypropylene 90% shading net (Bartex 90%, Artes Politecnica Srl, Schio, Vicenza, Italy) was placed over the trees 30 d after full bloom (DAFB) and removed after 8 d. Chemical thinning (1-Naphthaleneacetic acid 12 μg ml^{-1} + 6-Benzylaminopurine 120 μg ml^{-1}) was applied 14 DAFB to the control trees only.

Parameters recorded

Full bloom occurred on 9 April 2007. After 10 d, ten clusters of fruitlets were selected per tree, each bearing ≥ five fruitlets that appeared to have set. At 4-5 d intervals, fruit drop was determined by counting the number of fruit per cluster until the end of fruit drop. Sixteen determinations were made between 10 - 80 DAFB. Fruit growth (diameter in mm) was determined on a sample of 60 fruit per treatment at intervals from 4 - 15 d (more frequently early in the season). At each recording of fruit growth, a destructive sample of 32 fruit was taken from adjacent trees. This allowed us to perform a regression analysis between fruit diameter and fresh weight

(FW) which was used to follow the FW of those fruit measured throughout the season. From these data, absolute and relative fruit growth rates were calculated.

To monitor changes in whole-canopy gas exchange and fruit diameter more precisely from 27 - 47 DAFB, three trees and six fruits were chosen for each treatment. Hourly measurements of fruit diameter were done using custom-built fruit gauges (Morandi *et al*., 2007). Whole-canopy net CO_2 exchange rates (NCER) were determined on the same trees using a "balloon system" (Corelli Grappadelli and Magnanini, 1993; 1997). For each tree, NCER was measured four-times h^{-1}. In order to raise the number of replicates, NCER values obtained from all three plants, for all measurement days, were averaged over the two treatments. The fraction of light interception by the canopy, computed as (1- PAR below canopy) PAR^{-1}, was recorded for the same trees on 13 June 2007, under clear sky conditions, using a custom-built linear light scanner as described by Giuliani *et al*. (2000) featuring 48 PAR sensors spaced 5 cm apart. The light interception data were used to compute NCER per unit of intercepted light (i.e., specific NCER).

At harvest (9 August), total fruit numbers, yields per tree, and trunk diameters were recorded. Twelve fruits were sampled at random from each tree and various fruit quality parameters were measured: fruit size, fruit weight, colour (using a CR200 Chroma-Meter Colorimeter; Konica

Minolta, Sensing, Japan), firmness (TR model 53205, digital firmness tester; Turoni, Forlì, Italy), titrable acidity (Titromatic, compact titrator; Crison, Barcelona, Spain), and soluble solids concentration (PR32, digital refractometer; Atago, Japan).

Statistical analysis

Data were analysed as a completely randomised design (CRD), with each trees as a replicate. The percentage fruit drop data were first arcsine transformed. Specific NCER data for the two treatments were regressed against each other for three periods: before, during, and after shading.

RESULTS

Fruit growth

Generally, 'Gala' fruit were similar in FW between the two treatments until 32 DAFB, except at 21 DAFB (corresponding to 6 d after the application of the chemical thinners), when fruit from the chemical treatment showed a reduction in growth rate. In the same way, starting 6 days after shading began (i.e., 36 DAFB), shaded fruits had lower FWs until 60 DAFB. After that date, fruit FWs were always comparable in both treatments until harvest (Figure 1).

RGR and AGR values in chemically thinned fruit were lower than in the shaded treatment 17 and 21 DAFB, respectively. After completion of fruit drop caused by the thinning agent, fruit in that treatment showed increased

RGR and AGR from 32 to 38 DAFB. Likewise, after shade removal, the AGR and RGR of these fruit equalled and sometime overcame the values recorded on chemically thinned fruit (Table I).

Fruit drop

Fruit abscission started between 2 - 3 WAFB (Figure 2). More fruit fell from chemically-treated trees between 21 - 44 DAFB. The shading treatment caused fruit drop later (from 29 to 51 DAFB). At 7 WAFB, fruit drop almost stopped and both treatments achieved the same levels of fruit thinning (58.4% *vs.* 56.3% for chemical and shading treatments, respectively).

Hourly fruit growth

Hourly AGR (Figure 3) appeared to be similar for both treatments. Changes in fruit diameter before shading showed a similar pattern between treatments. From midnight, the AGR decreased becoming negative at sunrise (approx. 07.00 h) and reaching a minimum at approx. 14.00 h. Subsequently, the AGR started to increase again, becoming positive at approx 16.00 h and reaching its maximum value around sunset (19.00 h).

In the shaded treatment the fruit growth pattern changed, inducing smaller diameter increases and diminished shrinking. This modification in growth pattern increased with time under the shade cloth, resulting in almost no fruit growth at the end of the shading period. After shade removal, fruit in both treatments resumed similar growth patterns. The daily RGR rates

(Figure 4) at 27 DAFB were 15 and 19 μm mm^{-1} d^{-1} for chemical *vs.* shaded respectively. At 36 DAFB (during shading), the RGR of shaded fruit was more than ten-fold lower than control (8.3 *vs.* 0.5 μm mm^{-1} d^{-1} for chemical *vs.* shaded fruit, respectively). Three days after removal of the shading, fruit from both treatments were again comparable in the pattern and magnitude of hourly changes in diameter.

Canopy gas exchange rates

The net CO_2 exchange rate increased quickly after sunrise, reaching a maximum at 09.00 h, after which a gradual decrease occurred until 18.00 - 19.00 h, when respiratory losses became greater than the amount of C fixed, leading the C-balance to negative values in both treatments. Before the application of shading, and after removing the shading net, no differences were recorded between the two treatments. When the shading net was applied, a strong reduction in NCER was recorded during the daylight hours (Figure 5). Shaded trees reduced their net daily carbon gain between 06.00 and 18.00 h more than three-times compared to chemically treated trees, with an average assimilation rate of 33 g CO_2 d^{-1} (Figure 6).

Days chosen for whole canopy gas exchange measurements had a similar profile before, during, and after shading. Before and after shading, the highest rates of specific photosynthesis were recorded in the first hours of daylight (06.00 – 08.00 h), then it decreased until midday when the C-assimilation : intercepted light ratio stabilised at approx. 0.018 mol CO_2

mol^{-1} photons. During shading, plants under the net showed a specific NCER from 2- to 6-fold lower than the control (Figure 7).

Prior to placement of the shading net, the relation between control and shade-plant specific NCER was linear, with a slope of 0.97 and an intercept of 0.001 mol CO_2 mol^{-1} photons. The linear relation was maintained after removal of the shade net with slope and intercept of 1.08 and 0.0008 mol CO_2 mol^{-1} photons, respectively. Therefore, before and after the shade treatment, the relationship depicted a bisecting line (Figure 8). During the shading period, the linear relationship still persisted, however it was not a bisecting line and a slope of 0.46 was recorded (Figure 8).

Final fruit productivity and quality

The effect of chemical thinning or shading on crop load (4.17 or 4.08 fruits cm^{-2} trunk cross-sectional area; TCSA) and yield density (0.58 or 0.60 kg cm^{-2} TCSA) were the same. Fruit from the shading treatment had a similar weight, diameter, and background colour while their fruit sugar content, flesh firmness, titratable acidity and hue angle for skin colour were higher than those for chemically thinned fruit (Table II).

DISCUSSION AND CONCLUSIONS

Application of chemical thinners (NAA plus 6-BA) to 'Gala' caused a reduction in fruit growth 6 d after spraying, as reported previously for summer apple cultivars treated with NAA (Byers, 2003). The mechanism

by which hormone thinners cause an inhibition of fruit growth is not clear. Application of NAA inhibited carbon assimilation by 10 - 24% over a period of 2 weeks (Stopar *et al*., 1997), reduced stomatal opening (Snaith and Mansfield, 1984), and resulted in lower levels of reducing sugars and sorbitol in the fruit (Schneider, 1975).

A decrease in fruit growth was also recorded 7 d after the application of 90% shading. It is known that during this early period fruit are extremely sensitive to the lack of photosynthates caused by limitation of light or inhibition of photosynthsis (Byers *et al*., 1985) due to the competition for finite resources between many competing sinks. Moreover, the first few WAFB are important for subsequent fruit development (Lakso *et al*., 1989).

The final size of the fruit from both treatments would be insufficient for marketing from a commercial point of view. In both treatments, hand-thinning adjustments were avoided in order to reduce any unwanted influence on the results. Lack of the common commercial practice of hand-refinement of chemical thinning was probably responsible for this, and would likely be necessary for shaded trees as well.

During shading (30 - 38 DAFB), daily fruit abscission (Figure 2) varied between 1 - 5%. The decision to remove the shading net was taken when a sharp increase of fruit drop was detected between two consecutive determinations. The stress imposed by shading appeared as intense fruit abscission (23%) at 44 DAFB, shortly after shade removal, as observed by

Byers (1991). Lakso and Corelli Grappadelli (1992) suggest that the fruit abscission rate is related to their AGR and RGR. Before fruit drop increased, there was a decrease in growth rate (36 DAFB). After the heavy fruit drop had occurred, the remaining fruit increased their growth rates sharply (51 DAFB), probably as a result of resource availability. Byers (2003) showed selective abscission of the smallest fruit, which stopped their growth first, during, or soon after the shading.

The daily pattern of apple growth recorded was well known. Lang (1990) showed that the driving force for growth is fruit osmotic potential. After sunrise, increasing radiation and temperature and decreasing relative humidity (RH) promoted leaf transpiration which resulted in a lower leaf water potential than in the fruit.

In apple, active phloem unloading into the fruit takes place, and assimilates are compartimentalised within the vacuoles. Water can be lost from fruit by transpiration and/or by xylem backflow towards the leaves, causing fruit shrinkage. At night, as temperatures decrease and the RH increases, transpiration ceases and the leaf water potential is restored to values higher than in the fruit. Under these conditions, both xylem and phloem unloading occur, causing fruit expansion exceeding that of the previous day. Therefore, the accumulation of water and photo-assimilates in the fruit is the result of a balance between incoming and outgoing fluxes.

This explanation of the mechanism of fruit growth explains the variation in fruit diameter shown by the hourly AGR in Figure 3. In low light conditions, the growth mechanism controlled by water potential gradients is modified slightly, fruit shrinkage and expansion are smaller, probably because of different micro-climatic conditions and because, as expected from other studies (Corelli *et al*., 1994; Tustin *et al*., 1992) shading reduces the export of photosynthates from vegetative organs and consequently fruit import. A reduction in light limits photosynthesis and, as less carbon resources are available to the vegetative and reproductive sinks, competition between them increases, to the disadvantage of the fruit (Byers *et al*., 1985; 1991; Corelli Grappadelli *et al*., 1990). Considering this response in fruit growth patterns, the question arises whether, in the early phases of apple growth, there may be a passive phloem inflow, which might be revealed by the shading treatment. With less shrinking at midday, fruit do not reach the lower water potentials and are therefore unable to take up phloem solutes and xylem water.

The whole-canopy gas-exchange data showed that, at the beginning of the experiment, the selected plants had a similar photosynthetic performance (Figures 5 - 7). The light environment affected net carbon uptake during the first weeks of fruit growth. The shading treatment reduced net carbon assimilation at both the tree and specific levels (Figures 5 - 7). Analysis of the linear regressions between the specific NCER values

of chemically- and shade-thinned trees showed similar slopes before and after the shading treatments, suggesting that the period of shading did not influence the photosynthetic performance of the whole canopy after shade net removal. Shaded trees reduced their specific NCER by approx. half (slope = 0.46) in comparison with control trees (Figure 8). Thus shading reduced the availability of photo-assimilates at a time when the stored C-reserves reach a minimum (near bloom), and early fruit growth depends primarily on current rates of photosynthate production (Hansen and Grauslund, 1973). Early in the season, spur leaf photosynthesis is inadequate to supply sufficient carbohydrates, so fruit growth is limited and the crop load is adjusted to the available carbon by fruit abscission. Shading 5 WAFB resulted in the retention of assimilates at sites of vegetative development and the reduction in carbon availability to the fruitlets (Tustin *et al*., 1992; Corelli Grappadelli *et al*., 1994).

The treatments did not significantly affect crop density or crop yield, measured as the number of fruit cm^{-2} TCSA or total fruit weight cm^{-2} TCSA, respectively; indicating the two thinning methods had the same efficiency. Fruit quality was equal, if not superior, in shaded compared to chemically-thinned fruit, as reported in 'Golden Delicious' and 'Elstar' in Switzerland (Widmer *et al*., 2007). In conclusion, this indicates that, as a thinning method, shading has the potential to be used by apple growers to

reduce crop load and that shading acts selectively against slower growing fruit, which have a reduced potential for growth and quality.

The data also showed that shading has similar effects on fruit growth compared to earlier-acting chemical thinners. It may be that plant growth regulators can create a carbon unbalance against fruit earlier in the season than would occur under normal circumstances. The carbon-balance model proposed by Lakso (1999) indicates that at approx. 30 DAFB, the carbon balance in apple trees may be negative, because of the high demand of actively growing sinks, while the source leaves are still insufficiently developed. At 30 DAFB, others have reported the effectiveness of inhibition of photosynthesis, in addition to the data reported here.

The duration of shading required to be effective remains difficult to define. Data such as those reported here can be used to validate carbon-balance model predictions of the extent and intensity of carbon deficit caused by shading, based on climatic data. The goal would be to determine, from a predictive modelling standpoint, the time for shade removal for optimum efficacy.

FIGURES and TABLES

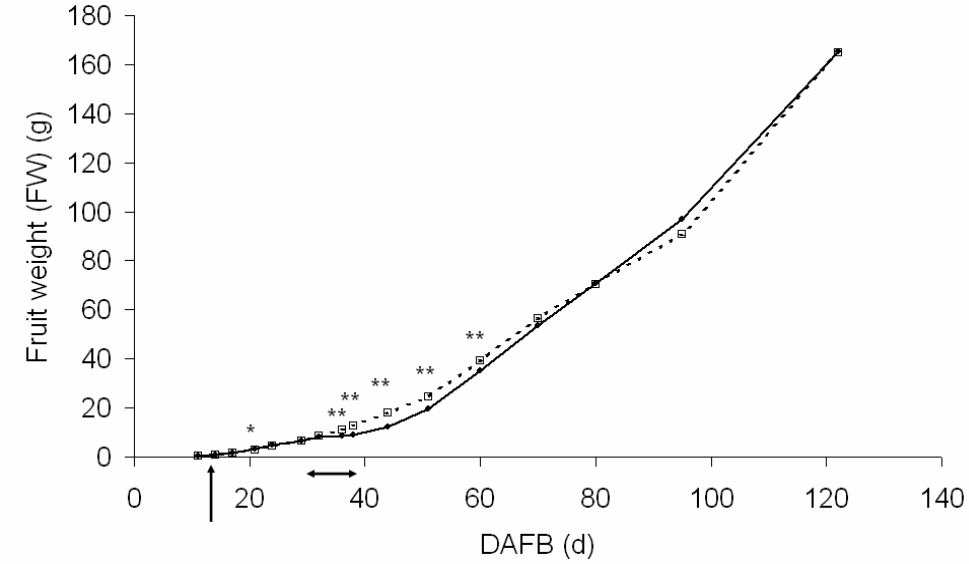

Fig. 1. Fresh weight (FW) of fruit growing on chemically-thinned (open square, dashed line) trees or trees thinned by shading (black dot, solid line). The vertical arrow indicates the date of chemical thinning. The horizontal arrow indicates the start and end of shading. *, **, significant at $P \leq 0.05$ or $P \leq 0.01$, respectively.

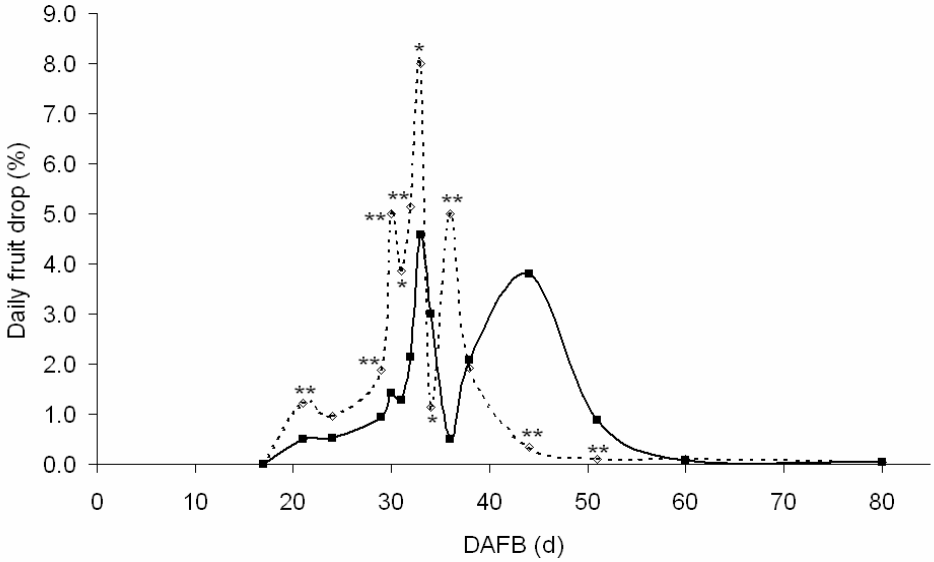

Fig. 2. Daily fruit drop (%) in chemically thinned trees (open diamond, dashed line) or by shading (black square, solid line). *, **, significant at $P \leq 0.05$ or $P \leq 0.01$, respectively.

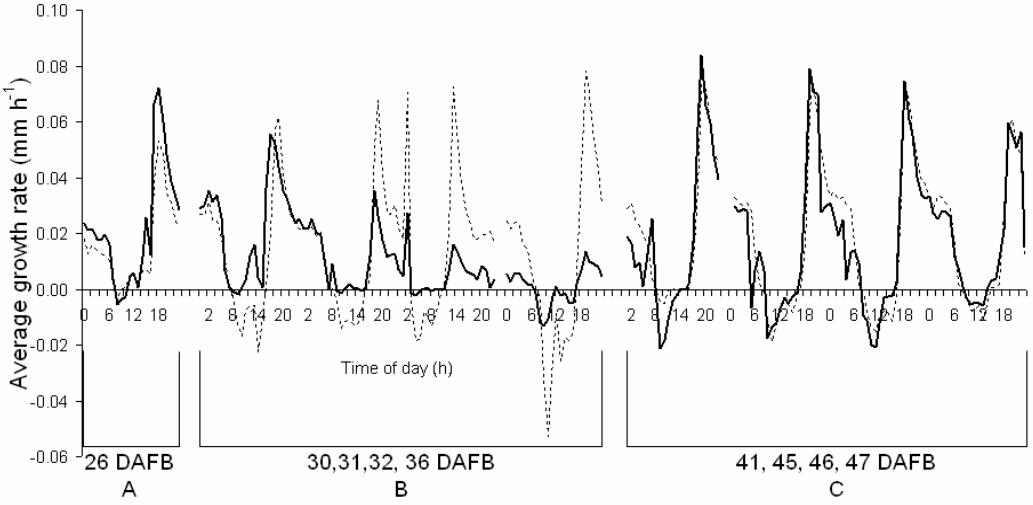

Fig. 3. Fruit hourly absolute growth rate measured by electronic gauges in trees thinned chemically (dashed line) and via shading (solid line) at different days (A = before shading; B = during shading; C = after shading). Each line represents the average of 6 fruit.

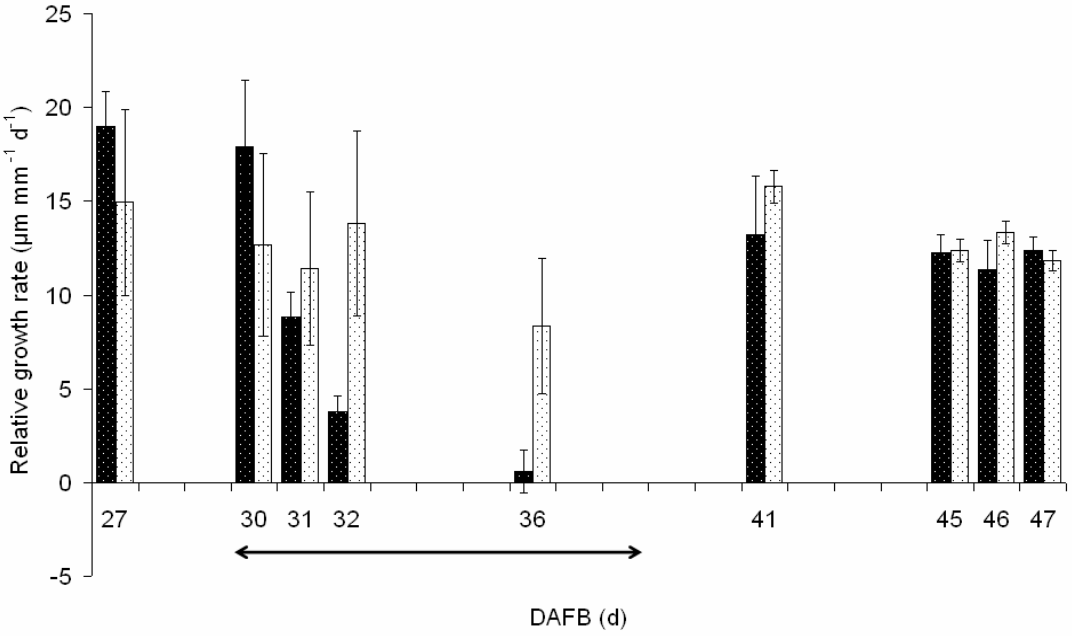

Fig. 4. Daily relative growth rate (RGR) of apple fruit measured by electronic gauges in chemically-thinned or shaded trees (white and black columns, respectively). The horizontal arrow indicates the start and end of shading. Vertical bars represent ± standard error.

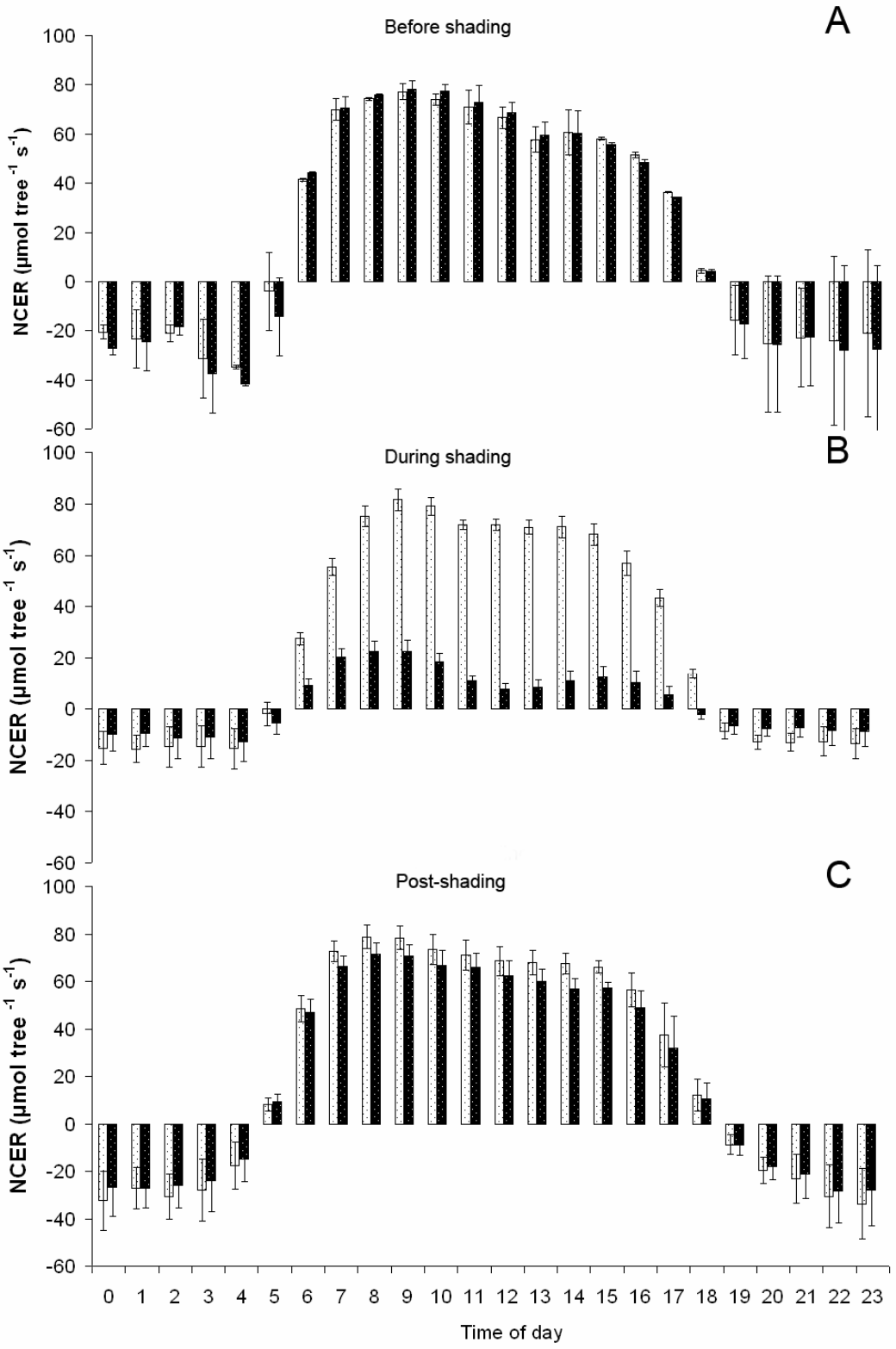

Fig. 5. Total hourly net carbon exchange rate in shaded apple trees (black columns) or chemically-thinned trees (white columns) at different days (panel A = before shading; panel B = during shading; panel C = after shading). Each bar represents an average of four hourly measurements on three trees per treatment ± standard error.

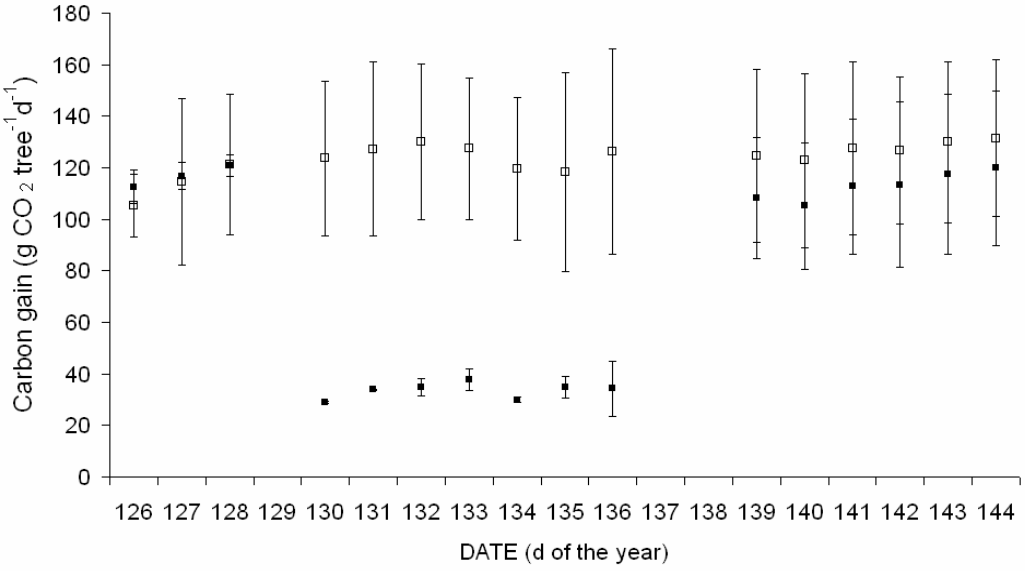

Fig. 6. Daily carbon gain (g CO_2 $tree^{-1}$ d^{-1}) in both treatments from 06.00 h to 18.00 h for shaded trees (solid black squares) and chemically-thinned trees (open squares). Each point is the average of three trees ± standard error.

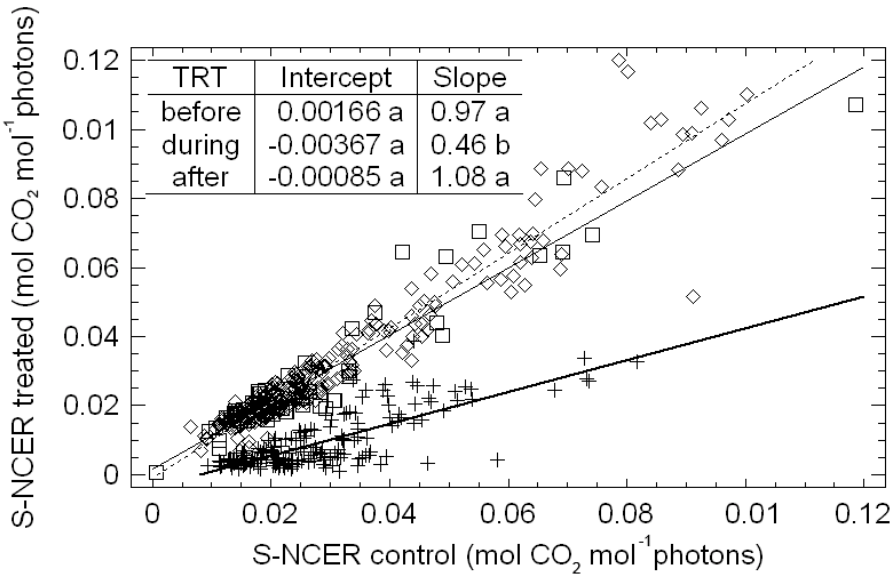

Fig. 8. Linear regression analysis between specific NCER values before shading (squares; thick line), during shading (crosses; bold line) and after shading (open diamond; dashed line). The insert reports the results of statistical analysis for the slopes and intercepts of the three treatments. Values accompanied by different lower-case letters are statistically different at $P \leq 0.001$

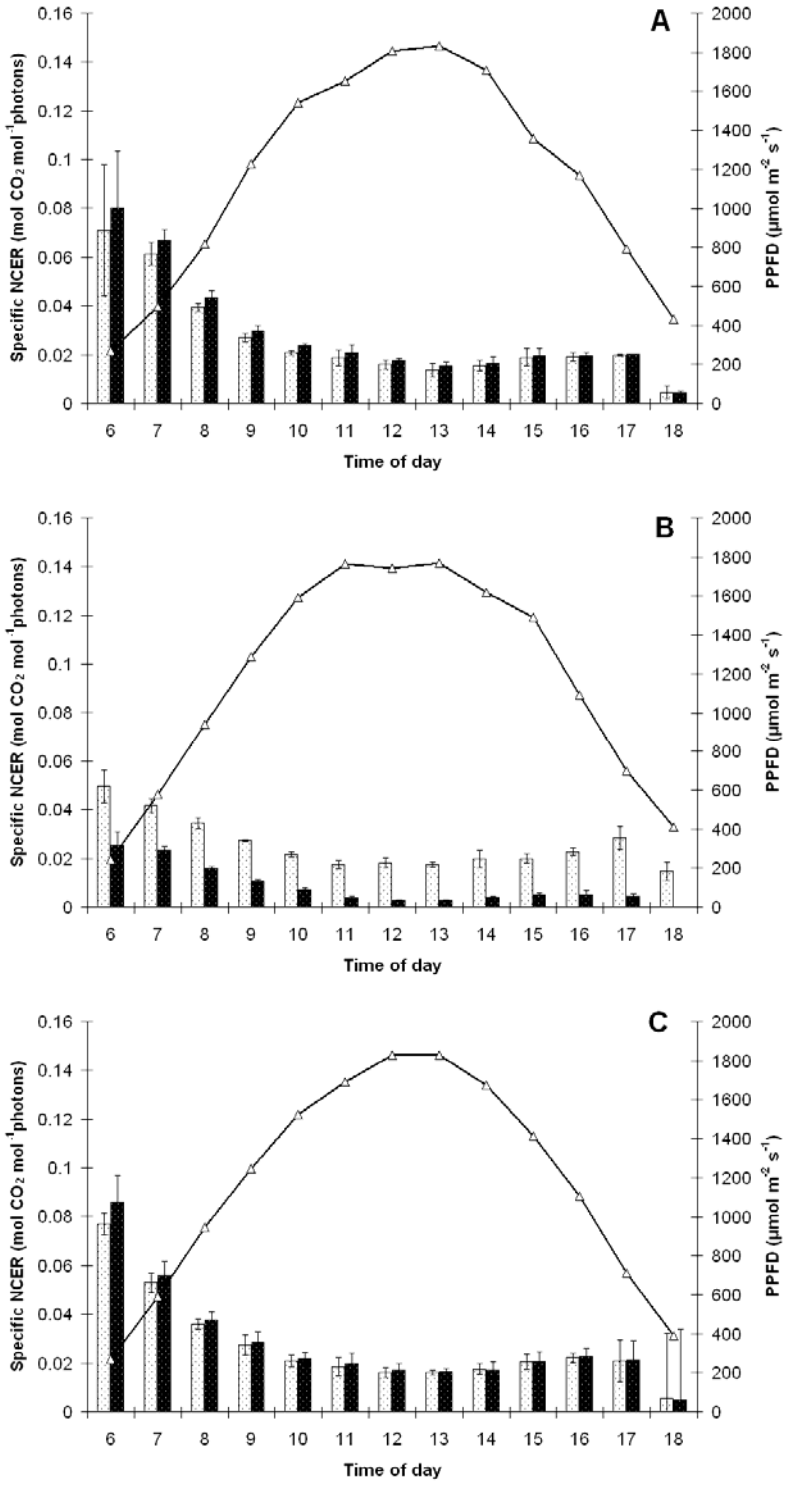

Fig. 7. Photons flux densities and specific NCER values in shaded or chemically-treated apple trees (black and white columns) from 06.00 – 18.00 h at different days (panel A = before shading; panel B = during shading; panel C = after shading). Vertical bars represent ± standard error.

TABLE I

Effects of shade and chemical thinning on growth rates and fruit drop in 'Imperial Gala' apple

Parameter	Thinning Treatment	DAFB										
		17	21	24	29	32	36	38	44	51	60	80
FRUIT	Chemical	0.0	4.9	7.7	17.1	31.1	50.3	54.1	56.1	56.9	57.7	58.4
DROP	Shade	0.0	2.0	3.6	8.3	13.3	21.7	25.9	48.7	54.9	55.6	56.3
(%)		ns[1]	**	**	**	**	**	**	*	ns	ns	ns
FRUIT	Chemical	1.54	2.93	4.55	6.40	8.40	11.2	12.9	18.1	24.7	39.2	70.2
WEIGHT	Shade	1.62	3.23	4.74	6.70	8.05	8.63	9.15	12.3	19.5	35.1	70.7
(g)		ns	*	ns	ns	ns	**	**	**	**	**	ns
AGR	Chemical	0.25	0.35	0.54	0.37	0.67	0.70	0.83	0.86	0.94	1.63	1.38
($g\ d^{-1}$)	Shade	0.28	0.40	0.50	0.40	0.44	0.18	0.30	0.50	1.00	1.73	1.71
		ns	*	ns	ns	*	**	**	**	ns	ns	**
RGR	Chemical	327	247	193	80.9	116	88.8	82.7	66.0	53.9	67.5	24.6
($mg\ d^{-1} g_{FW}^{-1}$)	Shade	370	264	162	88.5	65.3	25.1	43.1	58.4	86.5	90.2	32.3
		*	ns	*	ns	**	**	*	ns	**	**	**

[1] ns, *, **: mean comparisons within a column non-significant, significant at 0.05, or 0.01, respectively.

TABLE II

Effects of shade and chemical thinning on final production and quality of 'Imperial Gala' apples

Thinning Treatment	Crop Load (fruit cm^{-2})	Yield Efficiency (kg cm^{-2})	Fruit Weight (g)	Diam. (mm)	Sugar content (°Brix)	Flesh firmness (N)	Titrable Acidity (g l^{-1})	Background Color (hº)	Blush Color (hº)
Chemical	4.17	0.58	164.9	73.0	12.9	67.5	2.85	93.6	29.3
Shade	4.08	0.60	165.4	72.3	13.1	71.2	3.43	95.5	31.9
	ns[1]	ns	ns	ns	*	**	**	ns	*

[1] ns, *, **: not significant or significant at $P \leq 0.05$ or ≤ 0.01, respectively

Chapter III

MODELLING THE CARBON BALANCE OF APPLE TREES TO PREDICT THE TIMING OF SHADE REMOVAL

INTRODUCTION

The environmental sustainability of fruit process production is an important issue to deal with. Findings from previous studies showed that a strong but temporary light reduction could be used as a thinning method to induce fruit drop without chemical thinners. Based on the hypothesis that C-starvation may induce fruit abscission, shade is a possible way to thin apple trees. However, a method is needed to identify the length of shade application, because the strong reduction of incoming light for a very long time causes all the fruit to drop. Corelli Grappadelli and Musacchi (1994) proposed a method based on the observation of the abscission process while the shading was underway. Their aim was to detect a sudden change in the daily rate of fruit abscission, and to use this change as the decision making event in order to remove the shade. This approach had been devised and utilized in several studies of the effect of shading carried out in

the early 90's by Corelli Grappadelli (personal communication). However, further testing of this approach carried out in South Tyrol (Kelderer, personal communication) and in Zurich (Widmer, 2007) proved that, under those environmental conditions, the approach proposed by Corelli Grappadelli and Musacchi (1994) caused excessive thinning. Shorter lengths of shading were found to be effective, but in those lower vigor environments it appears as though the trees have less resources available and the abscission process proceeds faster, once it starts. Even in an environment such as that of Bologna, where the empirical approach utilized in the previous study worked, an approach which could eliminate the need for the daily monitoring of the abscission process would be desirable. As the assumption underlying this method of fruit thinning is that of a reduction in carbon availability to below a critical level, it was deemed that a modelling approach could be useful to simulate the carbon balance variation. In the past several models were developed in order to simulate apple tree photosynthesis and carbon balance (Thorpe et al., 1978; Seem et al., 1986). These models require a significant amount of input data, in order to provide the result. To overcome this difficulty, a simplified whole tree carbon balance and dry matter production model has been proposed and refined over time (Lakso et al., 1999, 2001, 2002), which requires as inputs only daily max and minimum temperatures, and daily light interception. The model has a day time step, which makes it potentially quite useful for

accomplishing the determination of the time of shade removal. Although it is a “simplified” model, the Malusim model is a compound of several submodels, each trying to simulate one specific part of the tree carbon balance. The growth of shoots is for example based on temperature accumulation, but this is critical to setting the photosynthetic potential of each shoot, as under low temperatures shoots are expected to grow slowly and to develop photosynthetically competent leaves at a slower pace. All tree parts are accounted for, although root respiratory losses with a greater degree of approximation than other parts, given the difficulty of studying root behaviour under field conditions. All the model subroutines therefore depend on parameters, which are set at some values depending on factual observations carried out in the place where the model has been developed. This aspect may seriously limit the usefulness of a model, if it is tried to be adopted in a different environment.

Measuring whole-tree gas exchanges can provide an useful benchmark against which to assess a model's capacity to predict tree carbon balance. The comparison between observed data and the model output could also be used to point the direction in which to change some of the model parameters, which was expected to be needed in the attempt of adapting the model to the environmental conditions of Bologna.

Corelli Grappadelli and Magnanini (1993; 1997) have suggested a method to measure whole-tree gas exchanges in the field. Their approach couples a

portable infrared gas analyzer (IRGA) to a "balloon" that encloses the tree, and measures the concentrations of CO_2 and water in the air entering and exiting the balloon. This system does not provide an estimate of root gas exchanges, so its output can only be considered relative to the canopy, but it is the single measure that is closest to the actual tree carbon balance currently possible.

This study reports gas exchange data collected on apple trees subjected to various lengths of shading during the post-bloom stage, and the carbon balance of the same trees, based on running the Malusim model with the environmental parameters (temperature and light) actually recorded during the same days. The results have been checked for their agreement, and capacity to explain the observed abscission levels.

MATERIALS AND METHODS

Plant material

The trial was carried out in an experimental orchard at the University of Bologna Experimental Farm in 2008, on 13-year-old trees of 'Imperial Gala' (*Malus* x *Domestica* Borkh.) apple on M.9 rootstock and 13-year-old trees of 'Golden Delicious' on M.9 rootstock. Trees were trained as free spindle and planted at a density of 2.857 trees ha^{-1}. The orchard was managed following standard fertilisation, irrigation and pest-management practices.

Experimental

Twelve Gala trees were chosen for uniformity of flowering and divided in two groups of 6 trees. The first group was covered from the 30 days after full bloom (DAFB) with a 90% shading net (Bartex 90%; Artes Politecnica SRL, Schio, Italy). Trees were uncovered in six different days, leading to six different shading duration (3, 4, 5, 6, 7. 10 days). The second group (used as control) was chemically thinned at 25 DAFB using 12 μg ml^{-1} 1-naphthalenacetic acid (NAA) + 120 μg ml^{-1} 6-benzylaminopurine (BAP).

Sixteen Golden trees were selected and divided in two groups of 8 replications per treatment, using single trees as replicate. The two treatments applied were (i) thinned using chemicals (control), or (ii) artificially shaded. Trees were covered 28 DAFB and the shading net was removed after 10 days. Chemical thinning [12 μg ml^{-1} 1-naphthalenacetic acid (NAA) + 120 μg ml^{-1} 6-benzylaminopurine (BAP)] was applied 25 DAFB to the control trees only.

Parameters recorded

Full bloom in Gala occurred on 9 April 2008, and on 7 April 2008, for Golden trees.

For two days prior to the shading treatment, during the period of shading, and until two days after the removal of the last shading, tree gas exchanges were continuously monitored on the six shaded Gala trees, using a whole canopy enclosure method. Overall, the daily carbon gain per tree was

determined from 28 – 40 DAFB using the enclosure system proposed by Corelli Grappadelli and Magnanini (1993; 1997), with a modification consisting in a computer controlled set of solenoid valves switching the air flows from the different balloons to the IRGA. A complete dataset per tree consisted in a reading of the CO_2 and water concentrations at the inlet and the outlet of the balloon. A reading of all 6 balloons required 15 minutes to complete, yielding 4 measurements per hour of NCER per tree.

Fruit drop in the balloon trees was determined by counting the number of small fruit fallen and gathered at the bottom of the canopy enclosure.

Twenty days after full bloom thirteen complete fruit clusters were selected per tree (in the controls only for Gala, and in the control and shaded for Golden). A cluster was considered complete when it bore at least five fruitlets that appeared to have set. At about 4 days intervals, fruit drop was determined by counting the number of fruit per cluster until the end of the fruit drop. Seven determination were made between 25 – 63 DAFB.

At harvest (16 September - Golden), total fruit number and yield per tree were recorded. Ten fruit were sampled at random from each tree for fruit quality parameters: fruit size, fruit weight, firmness (TR model 53205, digital firmness tester; Turoni, Forlì, Italy) and soluble solids concentration (PR32, digital refractometer; Atago, Tokyo, Japan).

Statistical analysis

Data were analysed as a completely randomised design (CRD), with each tree (Golden) as a replicate. The percentage fruit drop data were first arcsine transformed.

Malusim model

The Malusim model is a dry matter production and partitioning model developed by Alan Lakso at Cornell University using Stella software, which runs on the daily maximum and minimum temperatures and total radiation intercepted. Meteorological data from the site of the experiment were fed as inputs to Malusim, in order to simulate the daily carbon balance of the Gala trees, yielding six different simulations, according to the 6 shading durations applied.

The observed whole canopy gas exchange values were compared with the model predictions of the daily NCER, to assess the model's goodness of fit to existing conditions.

Before the simulation was carried out, some changes in the model parameters were made (orchard tree spacing; latitude, julian day of budbreak and full bloom, season length, shading period, etc.), in order to make Malusim more responding to local conditions.

RESULTS

Fruit drop

Fruit abscission in chemically thinned Gala trees started from 30 DAFB and almost stopped at about 45 DAFB (Figure 1), when shaded trees had a strong increase in fruit drop. In chemically thinned trees 53.7% of the fruit dropped. The six different shade treatments (3, 4, 5, 6, 7, 10 days shade length) had a similar initial fruit abscission pattern but longer lengths of shading induced fruit drop for longer, until different values were recorded when drop was complete (61.1, 72.8, 69.3, 69.5, 88.2 and 92.5% respectively). The dependence of fruit drop on length of shading appeared to be linear ($R^2 = 0.81$) (Figure 2).

In Golden, fruit abscission began four days after shading (Figure 3). The length of shading adopted was excessive, resulting in removal of 89.3% vs. 50.8% fruit for the shaded and the chemically treated trees, respectively.

Canopy gas exchange rates

Before the application of shading the NCER was about 70-100g. CO_2 $tree^{-1}$. Shading induced negative values in the C-balance of all trees until removal of the net (Figure 4). After shade removal, all the trees returned to NCER values similar to those observed prior to shading, with the exception of the two latest removals, which were uncovered at a time of low light intensities which caused a decrease in NCER of all the trees (Figure 4). The total net

carbon uptake from 30 to 39 DAFB ranged from -50 (shading for 10 days) to 500g tree^{-1} (shading for 3 days).

The loss of carbon gain (as computed based on the actual relative to the full light values) caused by shading was linearly related to the length of shading (R^2 = 0.74; Figure 5). The slope of the relation shows a daily carbon loss under the shading net of about 60g tree^{-1} per day.

Simulation output

The simulated daily carbon gain before shading is about 90g. CO_2 tree^{-1}. Under shade conditions NCER values ranged from -17 to 2g. CO_2 tree^{-1} day^{-1} (Figure 6). A goodness-of-fit analysis between observed and simulated daily CO_2 fixation integrals yielded a range of values between 0.76 and 0.95 (Figure 7). Nevertheless, in some cases a consistent overestimation of daily carbon gain was observed (trees shaded for 5 and 6 days; Figure 6).

A relationship was found between carbon gain loss and fruit drop (Figure 9). This relation obtained in a range of 300 – 800 g CO_2 lost (based on six trees) shows a 60% fruit drop corresponding to almost 300 g of CO_2 gain loss.

Final fruit productivity and quality

The number of fruit left on the Gala trees at harvest varied considerably, between 13 and 115. Despite this difference, a clear trend was observed of increasing fruit drop (as percentage) with increasing duration of shading.

The yield per tree in this experiment was not sufficiently compensated by larger fruit size, or weight. In Golden, shade decreased tree load to 64 fruit tree^{-1} and yield to 15 kg tree^{-1}, as opposed to 178 fruits and 27 kg tree^{-1} on trees chemically thinned.

Fruit from the shading treatment had a higher weight, diameter, sugar content, while flesh firmness was lower (Table 1).

DISCUSSION AND CONCLUSIONS

Fruit abscission in apple is not instantaneous, and this makes it difficult to achieve precise crop load control. Even in chemically thinned Gala trees, fruit drop started six days after the application of the chemical. In a similar way, the shaded trees showed a lag in their response: fruit drop started around one week after shade removal except for the tree shaded for 10 days. In this tree the thinning effect of shade appeared one day after net removal. The effectiveness of shading is quite high: all the shade treatments (3, 4, 5, 6, 7, 10 days shade length) caused higher final fruit drop compared to the chemically thinned trees. The two longest durations of shading (7 and 10 days) led to excessive fruit abscission levels.

This response was consistent with previous findings of the effect of strong light reduction, or alternative methods of photosynthesis inhibition, on fruit growth and retention (Zibordi et al. 2009). The fruit drop effect was related to the number of days of shading treatment, which further confirms the

carbohydrate starvation hypothesis, since a linear relation was also found between the decrease in carbon gain and the length of shading.

The simulations with the Malusim model show the reduction of daily carbon gain in low light conditions with acceptable accuracy, although in some trees they appeared to be underestimations of the degree of carbon loss achieved. However, these results could be considered satisfactory, given that most of the model parameters have not been modified from their settings for the upstate New York formulation.

The model for net carbon assimilation estimates could be an useful tool to regulate fruit crop in apple trees. It is currently being used as a tool to predict a fruit set potential for a given season, and to provide a guidance for chemical thinner dosages in New York State (A. Lakso, personal communication). However, even in that fruit producing region, the model is still not sufficiently precise to be possible to use it for the goal of this research. More precision is needed from the model, in particular the tendency to overestimate a carbon balance level should be reduced. More work and future parameterization could partially modify some Malusim equations and increase the accuracy of the simulations.

This work provides evidence for a physiological foundation to the mechanism leading to fruit drop, and provides two relationships that both confirm this mechanism, and suggest potential use in field conditions. The relation between length of shading and decrease in Carbon gain provides

support for the one showing that fruit drop increases with duration of shading. If the relation between final fruit drop and carbon gain loss due to shading will be confirmed, the model could be used as an helpful tool to decide the right time of shade removal.

Too much fruit fell from shaded Golden trees. This is in accordance with findings in Switzerland that show one week to be adequate for fruit thinning in this variety (Widmer, 2007). It is possible that 10 day shading caused such a strong reduction in carbon assimilation to cause a heavier fruit drop.

The excessive crop load reduction affected yields, which were diminished, and as a consequence the fruit quality parameters were in general better in the shaded than in the chemically thinned treatment.

In conclusion, this study shows has confirmed that shading has the potential to severely reduce crop load in apple. This could be used as a sustainable, innovative thinning approach, although the difficulty still exists of how to decide when to remove the shade, since fruit drop shows a lag in response, and waiting too long to remove the shade would result in overthinning, as was the case with Golden. The modelling approach attempted here was not sufficiently precise, although results from the simulations agreed with observed tree gas exchanges, at least as a general trend. While the model parameters should be modified for Italian conditions, two other relationships have been found, which might be

utilized to decide when to remove the shade: one between length of shading and carbon gain loss, and the other between shading duration and intensity of fruit drop. More work is needed to further test these relationships.

FIGURES and TABLES

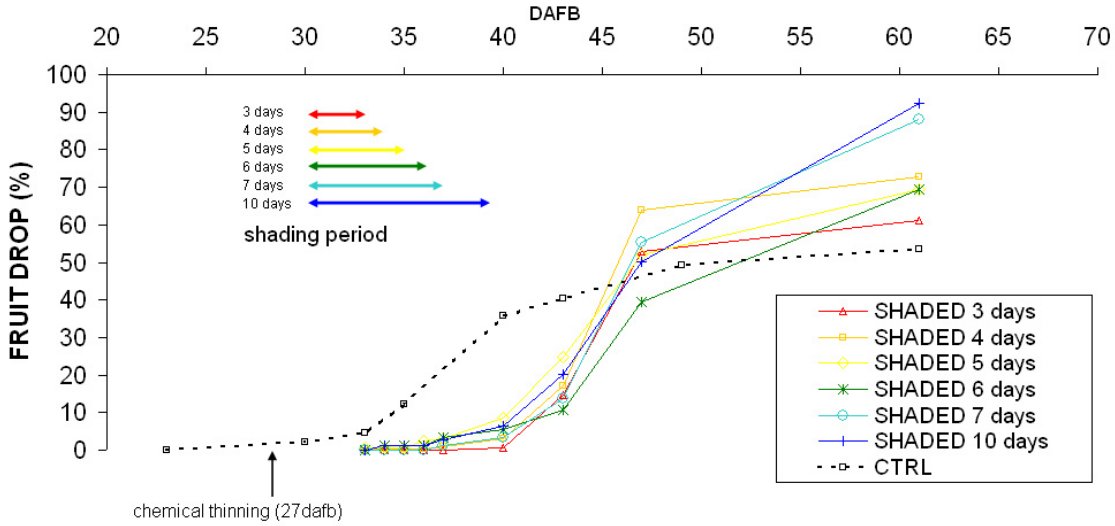

Fig. 1. Fruit drop evolution on plant shaded for 3 days (red line, open triangles), 4 days (dark yellow line, open square), 5 days (bright yellow line, open rhombus), 6 days (green line, asterisk), 7 days (cyan line, open circle), 10 days (blue line, cross) and chemically thinned (black spotted line).

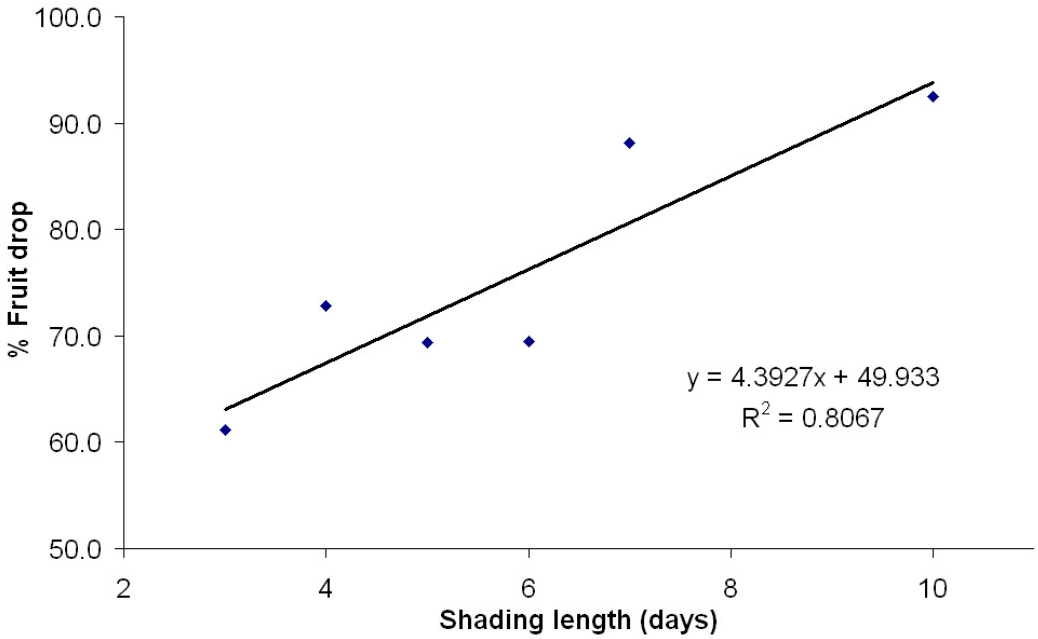

Fig. 2. Relation between shading duration and fruit drop on trees subjected at six different shading durations.

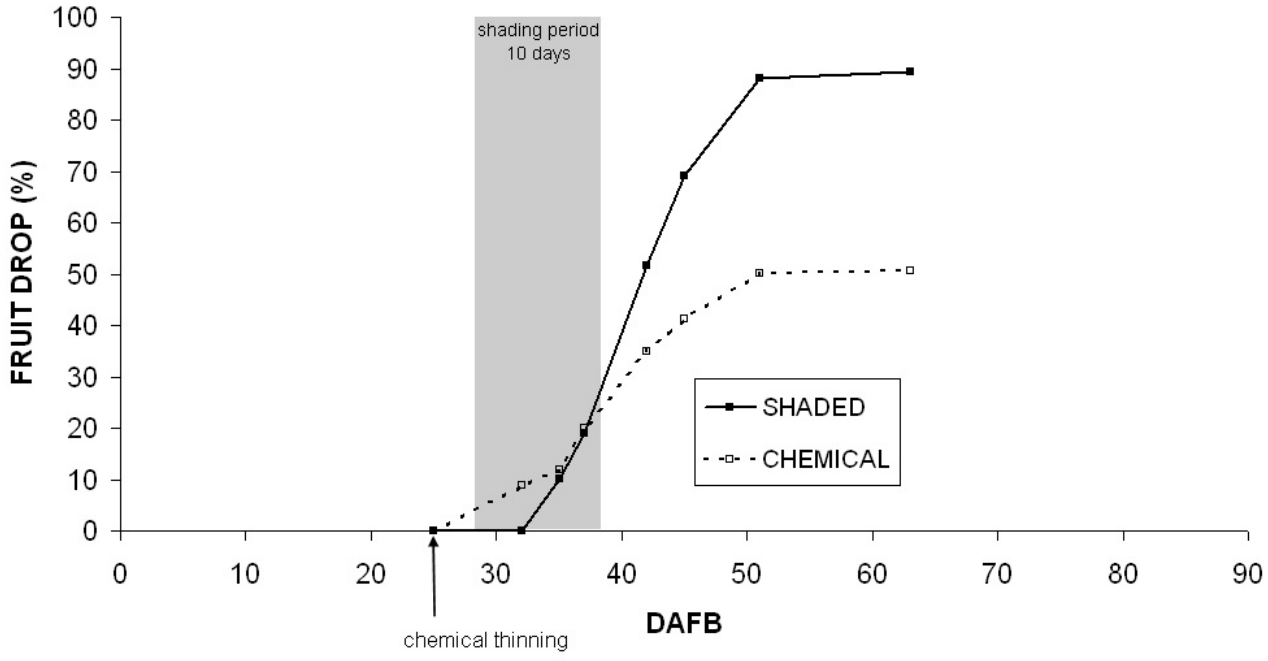

Fig. 3. Fruit drop seasonal pattern in Golden trees subjected at chemical thinning (black dashed line) and thinning via shading (black solid line). The gray area indicates the time of shading. Vertical arrow indicates the time of chemical thinner application.

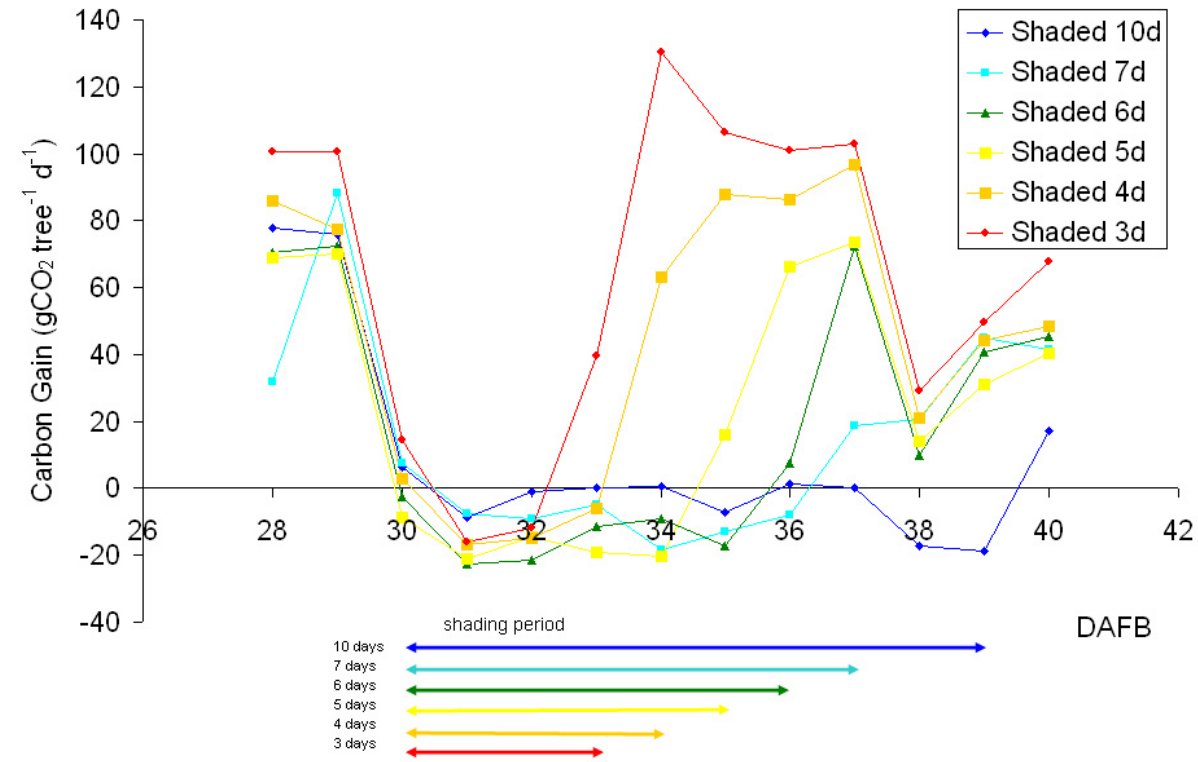

Fig. 4: Daily carbon gain measured on trees subjected at 3 (red line, circle), 4 (dark yellow line, square), 5 (bright yellow line, square), 6 (green line, triangle), 7 days (cyan line, square) and 10 (blue line, circle) days of shading.

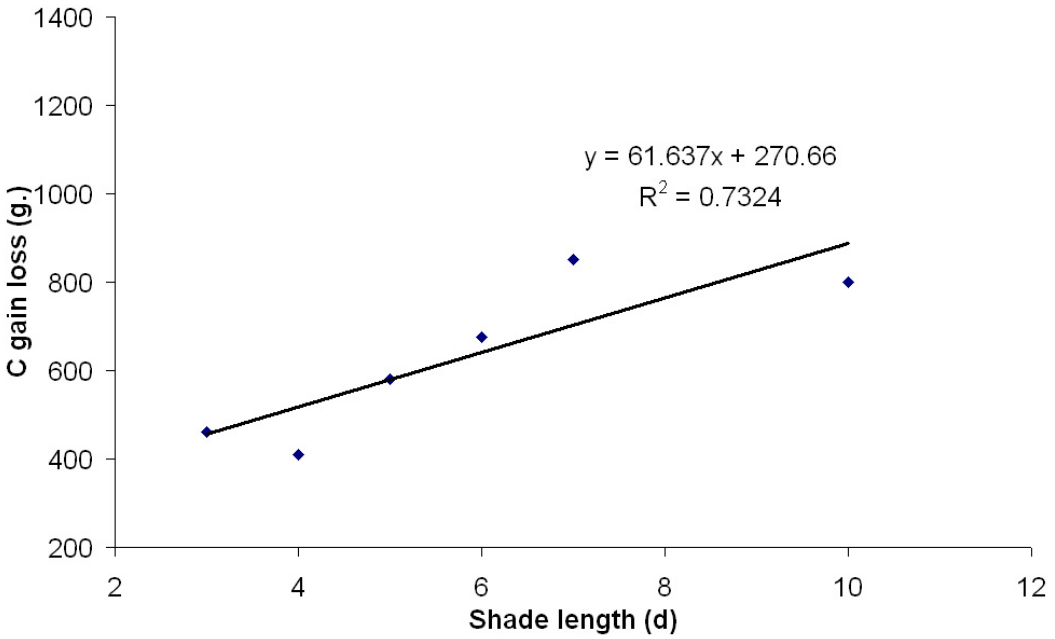

Fig. 5. Relation between shade length and loss of carbon gain on trees subjected at six different shading durations.

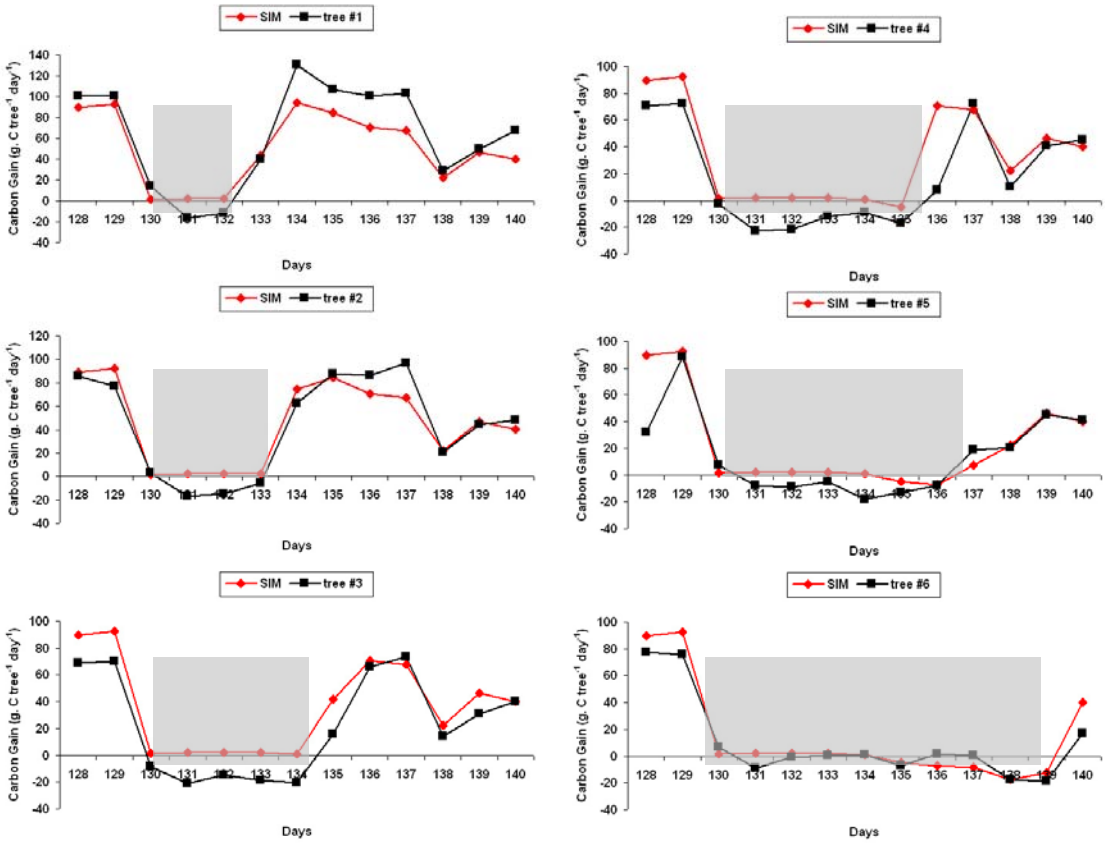

Fig. 6: Daily carbon gain pattern measured with the whole canopy gas exchange system (black line, square) and estimated with Malusim model (red line, rhombus) for each of the six shading duration. The grey area indicates the time of shading.

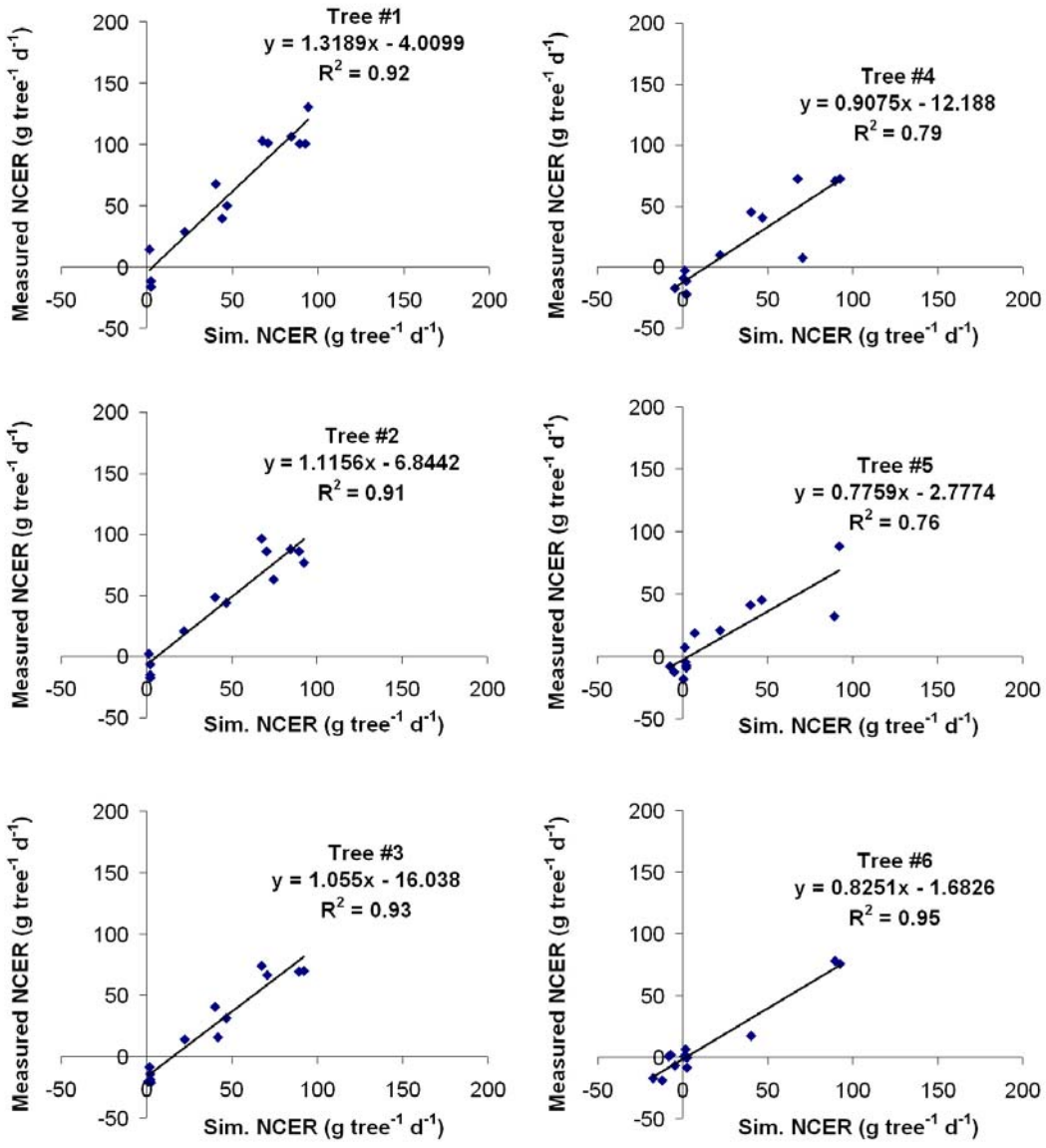

Fig. 7. Measured net carbon exchange rate (NCER) plotted against simulated NCER performed on trees subjected at six different shading durations.

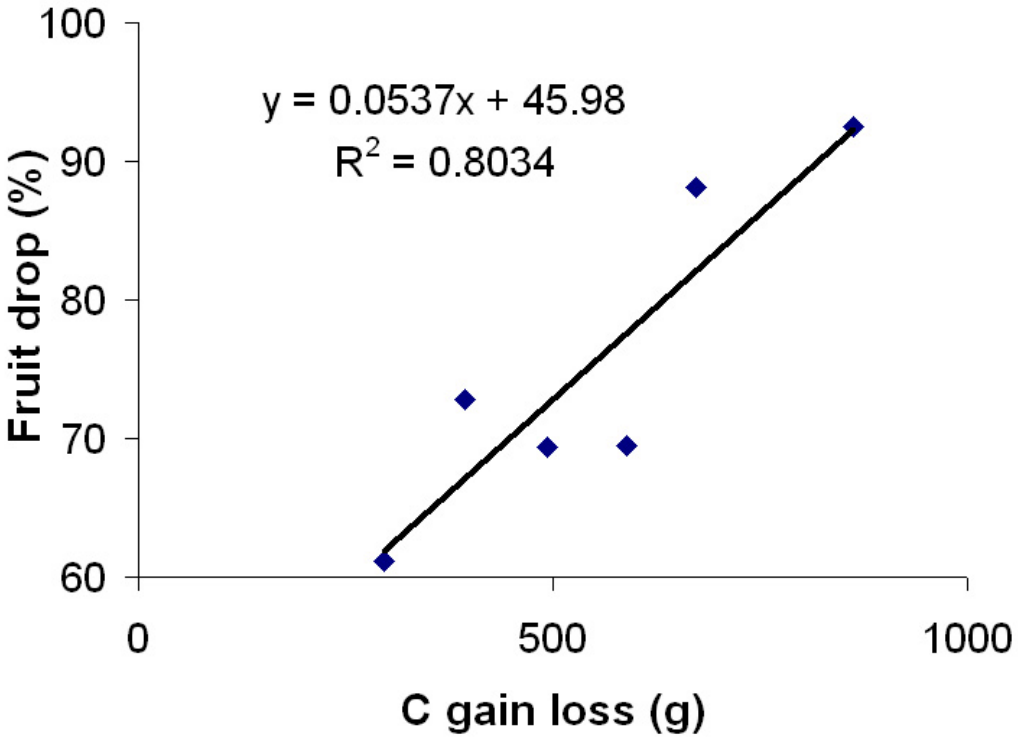

Fig. 8. Relationship between fruit drop and carbon gain loss (obtained by Malusim model)

Thinning Treatment	Fruit crop (fruit tree^{-1})	Yield (kg tree^{-1})	Fruit Weight (g)	Diam. (mm)	Sugar content (°Brix)	Flesh firmness (N)
Chemical	177.8	27.4	155.7	73.6	11.4	67
Shade	64.1	14.9	235.8	85.2	13.0	65
	**	**	**	**	**	*

[1] ns, *, **: not significant or significant at $P \leq 0.05$ or ≤ 0.01, respectively

Table 1. Principal fruit quality parameters measured for Golden apples chemically thinned and thinned via shade (shading durations: 10 days). Each data is the average of ten fruit.

Chapter IV

CAN THE REAL-TIME ASSESSMENT OF RELATIVE GROWTH RATE CONSTITUTE AN EFFECTIVE DIAGNOSTIC TOOL FOR DETERMINING LENGTH OF SHADING FOR FRUIT THINNING?

INTRODUCTION

While the thinning effect of shading during the post-bloom period in apple has been shown repeatedly, the difficulty has arisen of how to objectively determine when the shading should be removed, in order to achieve the best thinning results in apple. While an empirical approach previously tested has been found to be effective under the Bologna environment, (see chapter 2), this approach has not been found to be appropriate under less vigorous conditions, like the Alpine environments of South Tyrol and Northern Switzerland (Kelderer, personal communication; Widmer *et al.*, 2007). The search for a method applicable across the board has led this Dissertation to test the possibility of implementing the Malusim model

under Bologna conditions, to use the carbon balance of a hypothetical tree as a gauge of when sufficient stress would be applied to the tree. The results have been encouraging, but have also shown that a thorough parameterization for Bolognese conditions is a must, if the model were to be implemented. This leads back to the starting point, if one considers the amount of work needed for parameterization of a model, which would be necessary for every location in the world where it might be applied.

In the quest for a readily applicable diagnostic tool, the observations has been made in 2009 that, prior to reaching the peak of fruit abscission, the Relative Growth Rate (RGR) of surviving fruit showed an increase. The RGR can be considered as one of the earliest parameters of fruit growth that shows a response to environmental and physiological conditions (Lakso et al., 1989). This parameter indicates a growth rate normalized by the weight of the fruit itself and can increase in response to increased resource availability (Grossman and DeJong, 1995). Usually this parameter is characterized by decreasing values from the first week after full bloom until harvest.

A compounding factor to be accounted with is the fact that trees will have varying levels of natural crop load at the beginning of the season, depending on biennal bearing, for example. It would be nice to be able to account for this aspect as well, in devising a diagnostic tool capable of being implemented in a variety of orchard conditions.

This paper reports a study carried out in 2009 with the goal to explore the possibility of using a simple growth related parameter as a tool to decide the removal time of the shading net. The response was gauged on the level of starting crop load, which was set at three widely differing levels.

MATERIALS AND METHODS

Plant material

The trial was carried out in an experimental orchard at the University of Bologna Experimental Farm in 2009, on 13-year-old trees of 'Imperial Gala' (*Malus* x *Domestica* Borkh.) apple on M.9 rootstock. Trees were trained as free spindle and planted at a density of 2.632 trees ha^{-1}. The orchard was managed following standard fertilisation, irrigation and pest-management practices.

Experimental

Twenty-eight Gala trees were chosen for uniformity of flowering and divided in seven groups of 4 trees.

At 24 days after full bloom (DAFB) sixteen trees were subject to removal of entire spurs by hand to impose different crop loads. A medium crop load (Med) was set in half of the trees (8) by removing 50% of fruit. In the second half, 75% of fruit was removed, yielding a low crop load (Low). In each group four trees were thinned using shade cloth (-Med-shade; -Low-shade) and the remaining were used as control (Med-Ctrl; Low-Ctrl).

The remaining twelve trees were divided into three groups: chemically thinned (chemical), thinned by shading (-High-shade) and control (High-Ctrl).

"Chemical", "High-Ctrl" and "-High-shade" trees had an average of about 800 fruit per tree. "Med-Ctrl" and "-Med-shade" had an average of 360 fruit per tree. "Low-Ctrl" and "-Low-shade" had an average of 150 fruit per tree (Fig. 1).

Trees in the chemical treatment group were sprayed 33 DAFB using 12 μg ml^{-1} 1-naphthalenacetic acid (NAA) + 120 μg ml^{-1} 6-benzylaminopurine (BAP).

Twelve trees (-High-shade, -Med-shade and -Low-shade) were covered from the same day (31 DAFB) with a 90% shading net (Bartex 90%; Artes Politecnica SRL, Schio, Italy). Shading removal was decided looking at fruit RGR pattern: trees in "-High-shade" group were uncovered after 4 days whilst the net was removed from "-Med-shade" and "-Low-shade" after 8 days.

Parameters recorded

Full bloom occurred on 10 April, 2009. At 27 DAFB ten fruit clusters were selected per tree, each bearing at least five fruitlets that appeared to have set. Fruit drop was determined at appropriate intervals by counting the number of remaining fruit per cluster. A single fruit was marked for every cluster and its diameter carefully measured by calliper to study fruit

growth. Eight determinations of fruit drop and fruit diameter were made between 26 – 74 DAFB.

At harvest (10 August), total fruit number, distribution in fruit size classes and yield per tree were recorded. Five fruit were sampled at random from each tree and the following fruit quality parameters were measured: fruit size, weight, firmness (TR model 53205, digital firmness tester; TR-Turoni, Forlì - Italy) and soluble solids concentration (PR32, digital refractometer; Atago, Tokyo-Japan).

Statistical analysis

Data were analysed as a complete random design (CRD), with each tree as a replicate. The percentage fruit drop data were first arcsine transformed.

RESULTS

Fruit growth

No difference in fruit weight was observed until 33 DAFB between all the treatments (Fig. 2). From 38 to 42 DAFB all shaded fruit (High-, Med- and Low-shade) showed a reduction in growth rate. There were no differences in fruit growth between the chemical and the Ctrl treatments.

At 38 DAFB all the currently/previously shaded fruit had almost stopped their growth. The absolute growth rate (AGR) in these fruit was less than 0.1 g day^{-1} whilst control fruit were growing ten times faster (Fig.3). At 54 DAFB fruit from the Low- and -Med-shade treatments had similar AGR to

"Ctrl" and "chemical". On the same day -High-shade fruit had much lower AGR, but forty days after thinning , all fruit had similar absolute growth rate.

The relative growth rate (RGR) pattern showed some differences between treatments the first day of measurement but these differences vanished after two days. After five days of shading, higher RGR values were recorded for High- and Med-Ctrl fruit. Lower RGR values were recorded for the chemical and low-Ctrl fruit. All shaded fruit had RGR values close to zero.

Fruit drop

Fruit abscission started earlier (from 29 DAFB) in High-Ctrl trees (Fig. 5). On all subsequent dates, no more differences were recorded between treatments. Chemically thinned trees had almost the same fruit drop of Ctrl trees.

Fruit drop had a strong increase between 30 and 40 DAFB, then it stopped at about eight weeks after full bloom. More fruit fell from shaded trees but no differences were measured between different crop loads. Final fruit drop in shaded trees were: 97%, 100%, 97.5% for the High, Med and Low treatments, respectively.

At 54 DAFB all the fruit used to measure the growth of "-Med-shade" treatment abscised and this treatment was excluded from further analyses.

Fruit production and quality

Fruit from the -High-shade treatment were the largest (Fig. 6), at 210g. and 78 mm. Chemical and High-ctrl trees had the smallest fruit (less than 71 mm). The fruit size class distribution (Fig. 7) showed more than 70% of the fruit from High- Low-shade were bigger than 70 mm, whilst -Med-shade had only 58% of the fruit in this class. Chemical treatment and High- Med--ctrl had about 40 % of fruit bigger than 70 mm. No differences in weight (Fig. 6) between medium and low crop load were recorded.

-High-shade yielded one-third the fruit of the , High-ctrl trees (9 vs. 28 kg tree^{-1}). Low-ctrl trees had lower productivity than Med-ctrl (Fig. 8) but no differences were found between Med- and -Low-shade, that were the least productive trees (about 2.5 kg tree^{-1}).

Shaded fruit showed higher firmness than Ctrl apples, except for the Low-ctrl treatment. The least firm fruit belonged to the chemical treatment. A similar pattern was found for sugar content: fruit thinned by shade had higher Brix values (more than 13°) than “chemical” and “Ctrl” except for lower cropping trees.

DISCUSSION AND CONCLUSIONS

The results of this study do not lend themselves to an easy interpretation. Overall, the trees seemed to respond to all treatments in the least expected way. Shaded trees of different cropping levels showed no differences in

fruit growth and drop. The same situation was seen in Ctrl trees of all crop loads, where the rate of fruit growth and drop was not affected by widely different crop loads. It is likely that removing the entire spur caused a severe reduction in leaf area that the plants could not overcome. In fact, it is likely that the leaf/fruit ratio, albeit with different tree totals for the different crop loads, was not different among treatments.

While excessive drop was observed in the shaded treatments, the chemical treatment showed no effect on fruit drop nor growth: similar values were found between chemical and High-ctrl treatments (except for 33 DAFB). Shading had a very strong effect on fruit growth (fruit growth almost stopped as during the 2007 trial). This could be due to the competition for scarce resources between competing sinks (Byers et al. 1985). Comparing to 2008, shaded trees had a faster and earlier fruit drop (just two days after shading) and showed higher susceptibility to the treatment (100% or close in final fruit drop). Four days of light reduction in the High-shade treatment probably caused a very strong carbon gain unbalance and a consequent overthinning. Eight days under the shading net caused all -Med-shade fruitlet to drop, and very low productivity in Med and Low crop load trees. It is quite possible that these results, so widely different from experimentations in the previous years could be related to particular unfavourable weather condition during flowering. A response of this type occurring over a wide area of Uruguay in 2008 has been explained by the

Malusim model, which documented the negative effect of prolonged cloudy periods on tree carbon balance post-bloom (Lakso, personal communication).

Higher fruit loads in chemically thinned and High-ctrl caused a fruit size reduction if compared to shaded trees. On the other hand, High-shade trees, due to greater fruit drop, had an average production of only 9 kg tree^{-1}, only in part compensated by a size distribution shifted to bigger fruit (more than 70% above 70 mm). The same was found for sugar content and firmness, quite likely in response to the reduced crop loads.

It is difficult to write off one season's work to poor environmental conditions, but it appears as though the lack of response observed in 2009 was due to an experimental mistake (the removal of the entire spur, to facilitate the hand thinning), coupled to an unexpected response from the trees, which showed no effect of the chemical thinning treatment, while natural drop in non-thinned trees (Ctrl) or in shade-thinned trees was just overwhelming. This set of conditions has made it impossible to judge reliably the effectiveness of the approach based on the RGR as an indicator of the timing of shade removal. More work will be needed to define whether this approach is appropriate.

FIGURES

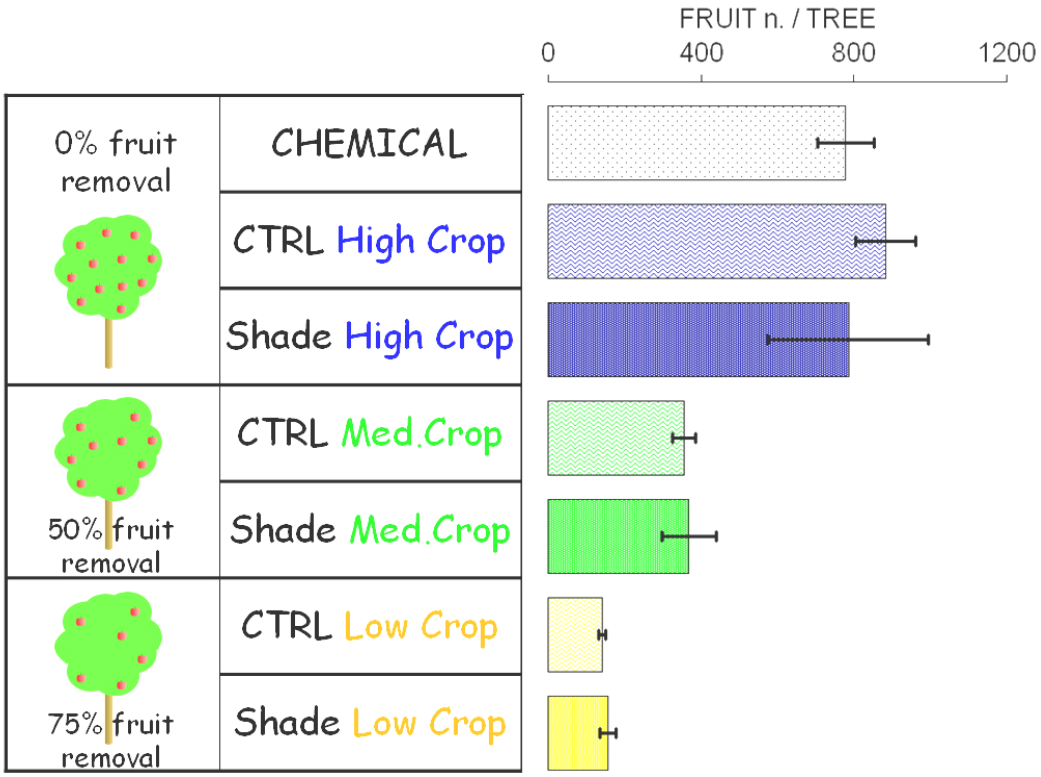

Fig. 1. Synthetical scheme of the experimental set. Depicted fruit number per tree means the crop load before the shading imposition.

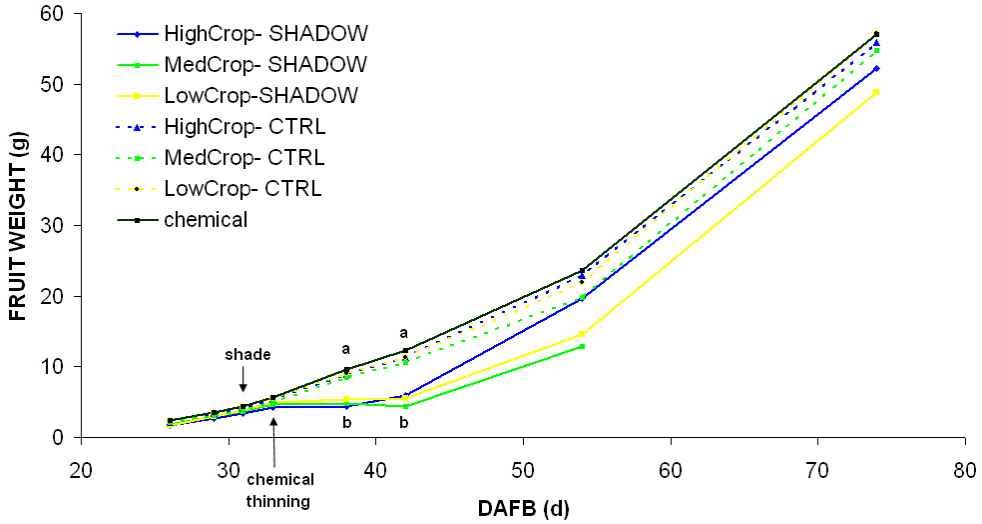

Fig. 2. Fruit fresh weight evolution within the first 10 weeks after full bloom computed for the all the seven treatments. Each point is the average of 40 fruit and those accompanied with different letters are different for $p \leq 0.05$.

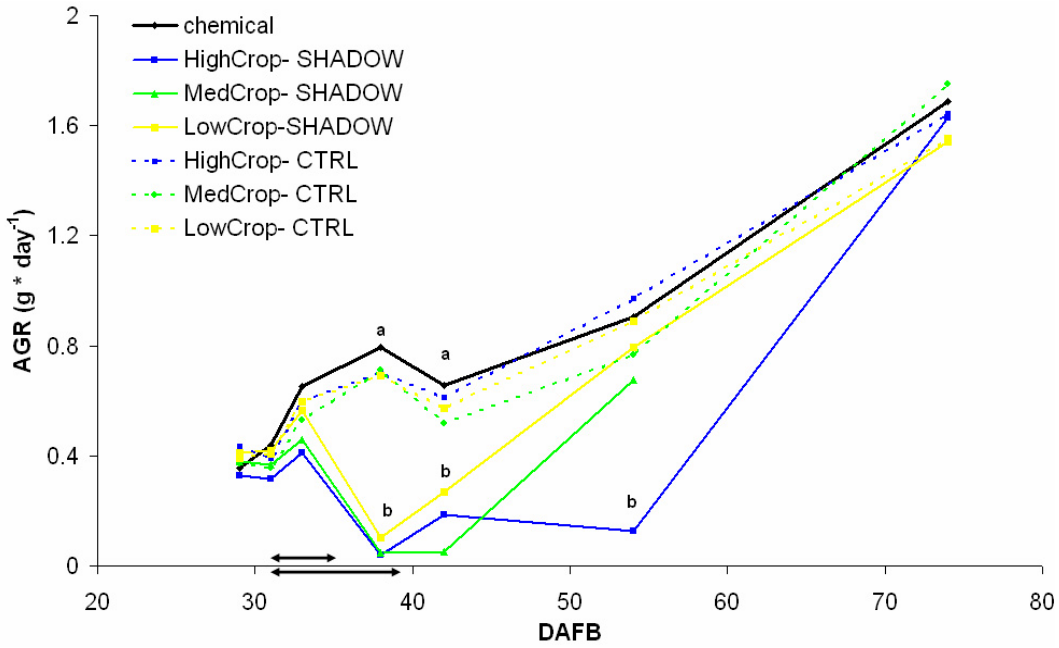

Fig. 3. Absolute growth rate (AGR) pattern within the first 10 weeks after full bloom computed for the all the seven treatments. Each point is the average of 40 fruit and those accompanied with different letters are different for $p \leq 0.05$. Horizontal arrows indicates the start and end of shading (4 and 8 days; "high-shade" and "med/low-shade" respectively).

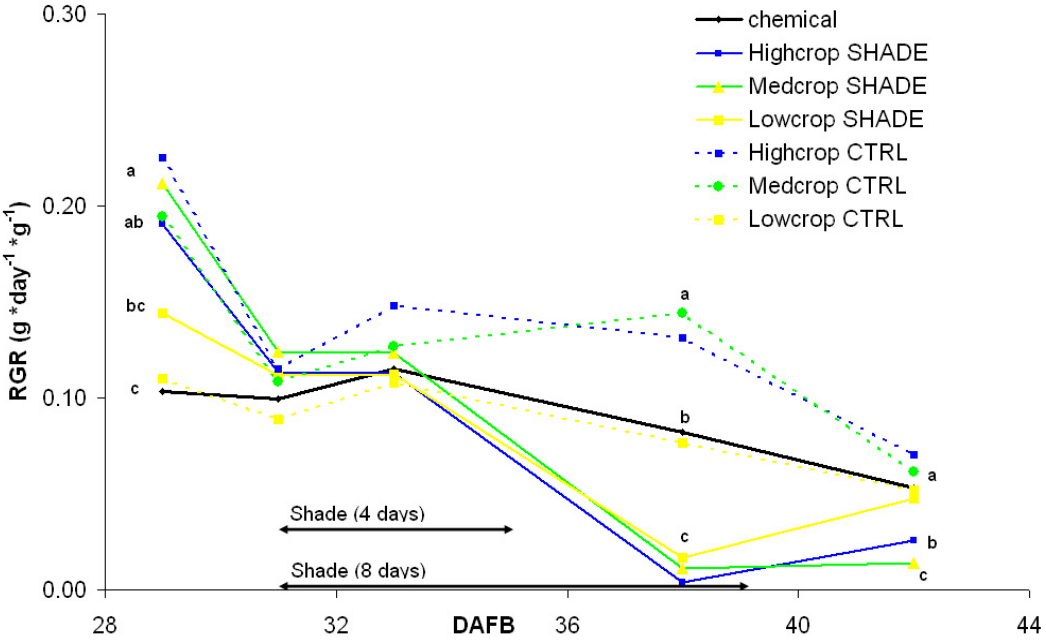

Fig. 4. Relative growth rate (RGR) pattern within the first 6 weeks after full bloom computed for the all the seven treatments. Each point is the average of 40 fruit and those accompanied with different letters are different for $p \leq 0.05$. Horizontal arrows indicates the start and end of shading (4 and 8 days; "high-shade" and "med/low-shade" respectively).

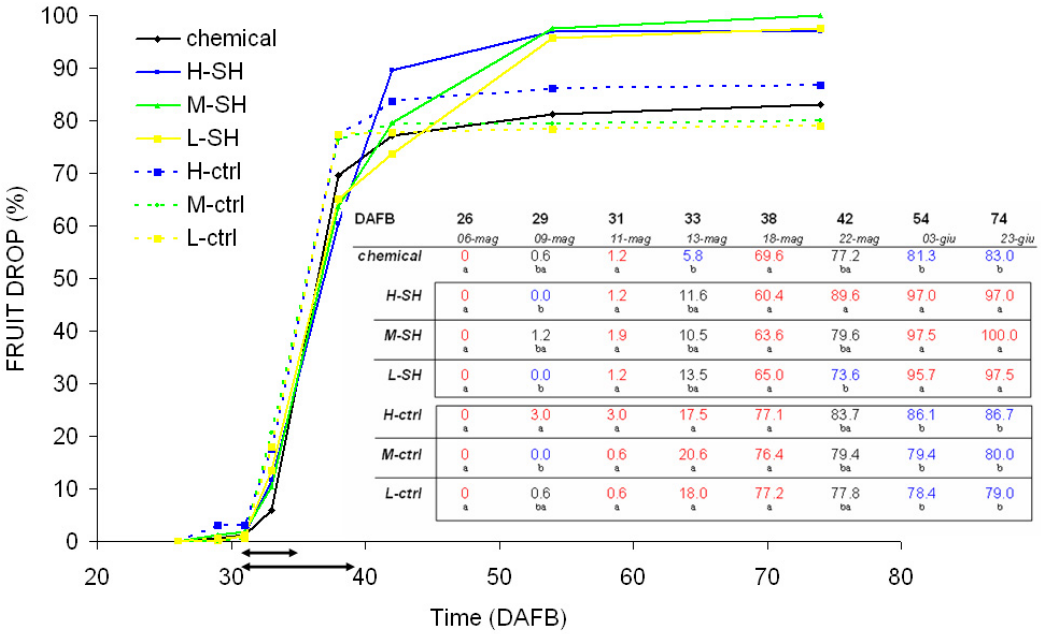

Fig. 5. Fruit drop pattern during the season computed for the all the seven treatments. Each point is the average of 40 fruitlets and statistical difference is represented on the attached table. Horizontal arrows indicates the start and end of shading (4 and 8 days; "high-shade" and "med/low-shade" respectively).

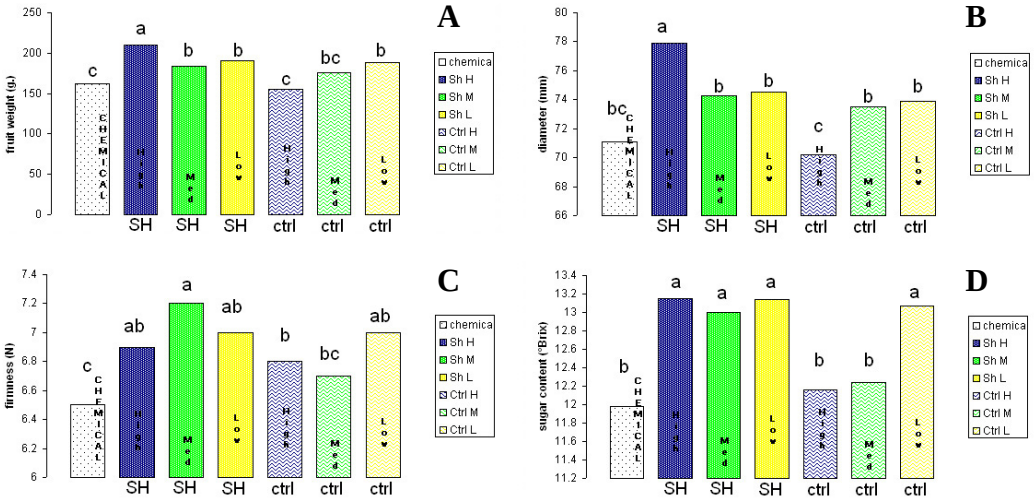

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Fig. 6. Fresh weight (A), diameter (B), firmness (C) and sugar content (D) measured at harvest on fruit belonging to all the seven treatments. Each bar represent the average of 20 fruit and those accompanied with different letters are different for $p \leq 0.05$.

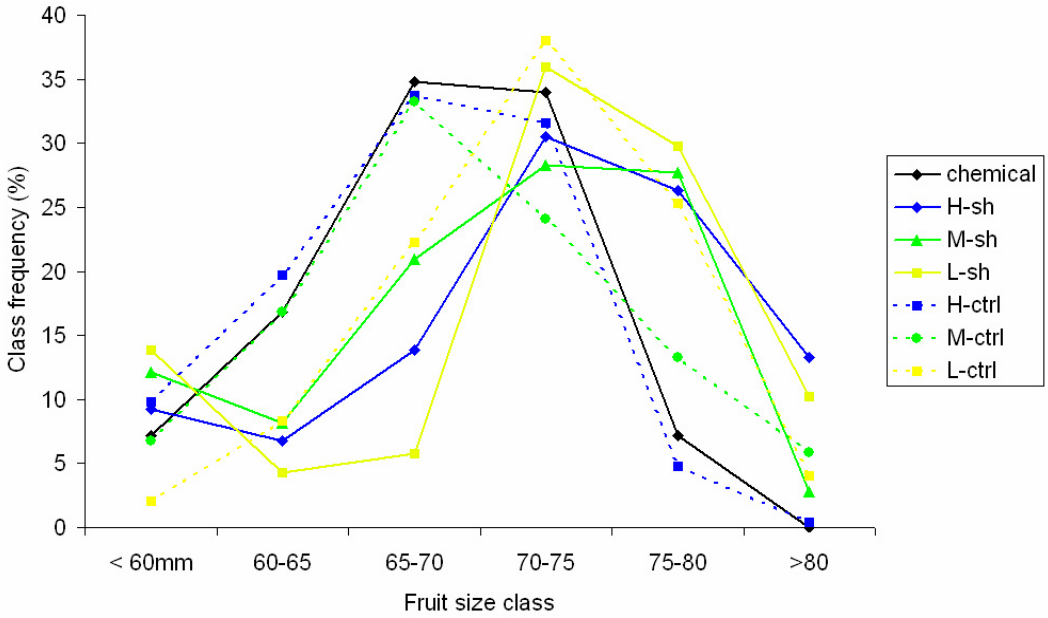

Fig. 7. Fruit size class distribution for all the seven treatments. Each point is the average of four trees.

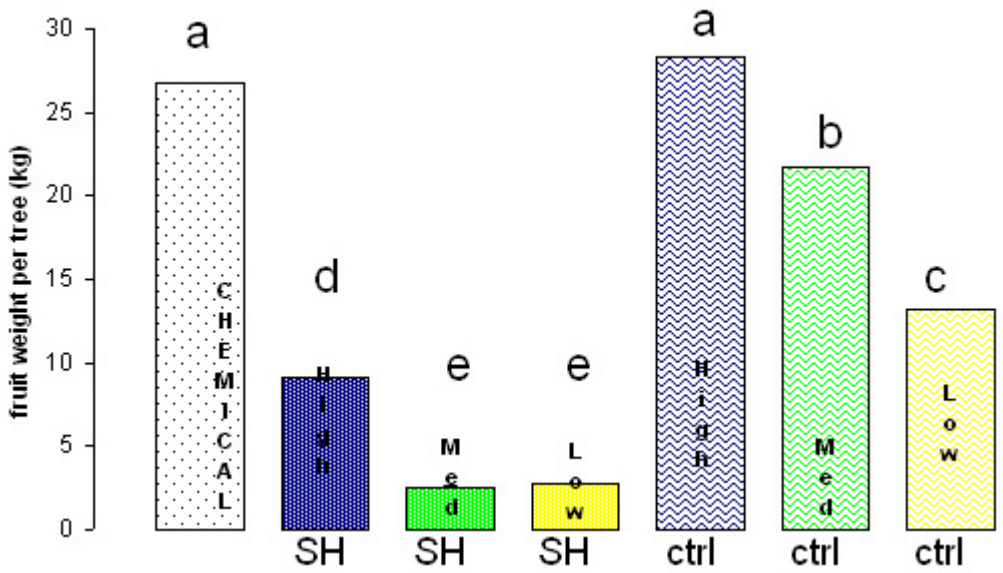

Fig. 8. Tree productivity for all the seven treatments. Each point is the average of four trees.

Chapter V

FINAL CONCLUSIONS

AND FUTURE PERSPECTIVES

The appropriate management of tree crop load is one of the key factor to produce highest possible yields and maximum quality fruit.

The modulation of light as thinning agent is an interesting approach which could help apple growers to reduce the use of chemical compound at least in the early reduction of crop load.

In 2007 the experimental work showed very good results following an empirical approach related to the daily rate of fruit abscission. Low light conditions reduced the availability of photo-assimilates and limited early fruit growth. Fruit abscission process adjusted crop load basing on available carbon. The C-starvation sensitivity seems to be strictly related to vigour environment. In fact trees grown in low vigour environment had stronger and faster abscission process which does not permit the use of this empirical system.

Searching for more widespread method, in 2008 we tried to determine from a predictive modelling standpoint, the time for shade removal for optimum

efficacy. A relation between length of shading and decrease in Carbon gain was found, supporting the one showing that fruit drop increases with duration of the shading. Simulations with Malusim model could be considered satisfactory, but more work needs to be done in order to increase the accuracy of simulations and test these relationship.

Unfortunately in 2009 our work was not sufficient to really test a fruit growth related parameter (RGR) to decide the removal time of shading net.

The shading approach and its influence on tree carbon balance still needs further work to be done but it could have a strong potential in apple trees thinning. Traditionally chemical thinners are characterized by unpredictable results and toxicity for the environment but now the modulation of light as a thinning agent could be used as alternative choice in early fruit crop regulation

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